Thieme

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Prof. Dr. Andreas Pascher

e1 Lectures and Posters

Lectures Session I Basic Hepatology (Fibrogenesis, NPC, Transport) Friday, January 29, 2021, 1:25 pm – 2:10 pm, Lecture Hall Virtual Venue

1.1 TGR5 (Gpbar-1) expression is downregulated in biliary epithelial cells in livers of PSC patients and in Abcb4-/- mice

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Introduction Primary sclerosing cholangitis (PSC) is a chronic cholestatic liver disease characterized by progressive inflammatory and fibrotic injury to the biliary tree. The localization of TGR5 in cholangiocytes and immune cells, the choleretic, and anti-inflammatory functions of the receptor suggest that TGR5 is important for the pathogenesis of biliary diseases. Aim of this study was to evaluate changes in TGR5 expression and localization in livers from PSC patients as well as from Abcb4-/- mice, which serve as an animal model for sclerosing cholangitis.

Methods TGR5 expression and localization in livers from PSC patients and Abcb4-/- animals were evaluated using immunofluorescence staining. TGR5 mRNA expression was analyzed in isolated intrahepatic and extrahepatic bile ducts from Abcb4-/- mice and WT controls by real-time PCR in relation to an endogenous housekeeping control. BECs (biliary epithelial cells) were isolated by microdissection or by FACS using positive selection for EpCAM from livers of 6-8 weeks-old animals. Abcb4-/- mice with genetic overexpression of TGR5 (Abcb4-/-/Tgr5-Tg) were generated and serum biochemistry as well as liver histology and immunohistochemistry were assessed.

Results Reduced TGR5 staining intensity was detected in BECs from liver tissue of PSC patients as well as in BECs of Abcb4-/- mice. This downregulation was not observed in BECs in liver tissue from PBC, NAFLD or viral hepatitis patients. No downregulation in TGR5 fluorescence intensity was observed in F4/80- and CD68-positive intrahepatic macrophages in Abcb4-/- livers. Analysis of isolated intrahepatic and extrahepatic bile ducts as well as in EpCAM+-enriched cells showed a significant decrease in TGR5 mRNA in Abcb4-/- mice as compared to the controls. Genetic overexpression of TGR5 in livers from Abcb4-/-/Tgr5-Tg

mice improved serum liver enzymes and biliary fibrosis compared to Abcb4-/- mice.

Conclusion TGR5 levels were significantly and specifically downregulated in bile ducts from PSC livers. Moreover, a significant reduction of TGR5 mRNA was detected in intrahepatic and extrahepatic bile ducts as well as in isolated BECs from Abcb4-/- mice livers. Genetic overexpression of TGR5 ameliorates the biliary fibrosis phenotype of Abcb4-/- mice.

1.2 Intravital dynamic and correlative imaging reveals diffusion-dominated canalicular and flow-augmented ductular bile flux

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Small-molecule flux in tissue-microdomains is essential for organ function, but knowledge of this process is scant due to the lack of suitable methods. We developed two independent techniques that allow the quantification of advection (flow) and diffusion in individual bile canaliculi and in interlobular bile ducts of intact livers in living mice, namely Fluorescence Loss After Photoactivation (FLAP) and Intravital Arbitrary Region Image Correlation Spectroscopy (IVARICS). The results challenge the prevailing “mechano-osmotic” theory of canalicular bile flow. After active transport across hepatocyte membranes bile acids are transported in the canaliculi primarily by diffusion. Only in the interlobular ducts, diffusion is augmented by regulatable advection. Photoactivation of fluorescein bis-(5-carboxymethoxy-2-nitrobenzyl)-ether (CMNB-caged fluorescein) in entire lobules demonstrated the establishment of diffusive gradients in the bile canalicular network and the sink function of interlobular ducts. In contrast to the bile canalicular network, vectorial transport was detected and quantified in the mesh of interlobular bile ducts. In conclusion, the liver consists of a diffusion dominated canalicular domain, where hepatocytes secrete small molecules and generate a concentration gradient and a flow-augmented ductular domain, where regulated water influx creates unidirectional advection that augments the diffusive flux. These findings overturn a common perception held for decades regarding the nature of biliary flux in the liver parenchyma, with implications on pharmacokinetics, anti-cholestatic drugs and therapy and basic liver physiology.

1.3 Role of autophagy in amiodarone-mediated hepatotoxicity

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Question Amiodarone, a widely used antiarrhythmic drug, can cause steatohepatitis, liver fibrosis and cirrhosis. The molecular mechanisms of amiodarone-mediated liver injury remain largely unknown. We therefore investigated amiodarone-mediated hepatocellular injury in patients with chronic heart failure, in primary hepatocytes and HepG2 cells.

Methods The apoptosis biomarker, caspase-cleaved keratin-18, was measured by ELISA in sera from chronic heart failure patients treated with or without amiodarone. Caspase activation was further analyzed in amiodarone-treated hepatocytes by western blotting and enzyme assays. Cell death was assessed by using a cell viability assay. Amiodarone-induced hepatocyte steatosis was detected by triglyceride staining and mRNA expression analyses of lipogenic factors. ER stress and autophagy were analyzed by western blotting of marker proteins.

Results Amiodarone-treated patients with chronic heart failure revealed significantly higher serum levels of caspase-cleaved keratin-18 compared to heathy individuals or patients not receiving amiodarone. We could demonstrate increased apoptosis in hepatocytes treated with amiodarone which was associated with lipid accumulation and ER-stress induction. Hepatocyte steatosis was accompanied by enhanced *de novo* lipogenesis which, after reaching peak levels, declined together with reduced ER stress. The decrease of amiodarone-mediated lipotoxicity was associated with protective autophagy induction. *Vice versa*, in hepatocytes treated with the autophagy inhibitor chloroquine as well as in autophagy gene (ATG5 or ATG7)-deficient hepatocytes, amiodarone-mediated toxicity was increased.

Conclusions Amiodarone induces lipid accumulation associated with ER stress and apoptosis in hepatocytes, which is mirrored by increased serum levels of caspase-cleaved keratin-18 in amiodarone-treated patients. Autophagy counteracts this amiodarone-triggered lipotoxicity and could provide a therapeutic strategy for protection from drug-induced liver injury.

Lectures Session II Clinical Hepatology, Surgery, LTX
Friday, January 29, 2021, 3:25 pm – 4:10 pm, Lecture Hall Virtual Venue

2.1 ARO-AAT REDUCES Z-AAT PROTEIN IN PIZZ PATIENTS AND LEADS TO IMPROVEMENTS IN CLINICALLY RELEVANT LIVER BIOMARKERS

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DOI 10.1055/s-0040-1721947

Background Homozygous PiZZ alpha-1 antitrypsin deficiency (AATD) is an autosomal co-dominant genetic disorder causing pulmonary and liver disease in children and adults. Wild type alpha-1 antitrypsin (AAT) is synthesized by hepatocytes and secreted into circulation to protect the lung during inflammation by inhibition of neutrophil proteases. The mutant Z protein (Z-AAT) misfolds and is retained in the hepatocyte rather than secreted. This triggers liver injury which can lead to cirrhosis and the reduced serum activity can lead to lung injury. Intracellular proteolysis pathways are activated in the hepatocyte to reduce Z-AAT accumulation, but liver injury still results in some individuals. ARO-AAT is a hepatocyte targeted RNAi therapeutic designed to silence expression of Z-AAT mRNA leading to reduced Z-AAT protein synthesis. Herein, we report initial results from Cohort 1 in the AROAAT2002 phase 2 clinical trial.

Methods 4 PiZZ AATD patients with liver fibrosis were enrolled to receive open label ARO-AAT 200 mg by subcutaneous injection at Weeks 1, 4 and 16. Patients underwent liver biopsy at Screening and Week 24. Assessments include safety (e.g. AEs, labs, spirometry and DLCO), serum and intra-hepatic Z-AAT, serum biomarkers of liver injury and fibrogenesis (e.g. ALT, GGT, Pro-C3) and transient elastography (FibroScan).

Results At Week 24, serum and total intra-hepatic Z-AAT decreased by 86-93 % and 72-95 % respectively. Three of four patients demonstrated reductions in intra-hepatic Z-AAT polymer at Week 24 with a range of 68-97 %. All four patients showed reductions in ALT and GGT from baseline to Week 24 ranging from 36-66 % and 43-58 % respectively. Liver stiffness (FibroScan) improved in all patients, with 3 of 4 patients demonstrating >20 % reductions at Week 24. Three of 4 patients demonstrated

reductions in the fibrogenesis biomarker Pro-C3 ranging from 31-51 % at Week 24. One SAE (EBV related myocarditis) was reported. No clinically meaningful changes in FEV1 were observed.

Conclusion ARO-AAT is the first investigational therapeutic to demonstrate reductions in intra-hepatic Z-AAT in humans. The associated improvement in clinically relevant biomarkers of liver disease is consistent with pre-clinical studies showing that Z-AAT accumulation is the causative factor in AATD liver disease. These data confirm that when Z-AAT synthesis is halted, endogenous proteolysis can clear accumulated Z-AAT with associated improvements in liver health.

2.2 IL-6 induces regeneration and angiogenesis in bile ducts and prevents complications after liver transplantation

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Question Bile duct (BD) damage leads to complications that are a major cause of morbidity and mortality after liver transplantation. Previous studies by our group have shown that donors' livers with damaged BD epithelia after cold storage are linked to an increased risk of developing these complications than livers with intact bile ducts. The molecular mechanisms leading to damage and healing are yet to be fully understood. However, in-vitro-studies suggest that IL-6 may play a striking role in bile duct regeneration as well as damage prevention. This study aims to examine this role using novel in-situ-hybridization methods combined with automated image analysis algorithms.

Methods During liver transplantation, specimens of donors' common bile duct were taken after cold storage and after reperfusion and fixated (FFPE). Following a HE-staining to determine the presence of 'major' bile duct damage, an in-situ hybridization for IL-6 mRNA was performed for each specimen, and the density of IL-6-positive cells in predefined areas (subepithelial/peribiliary glands) was analyzed using an image analysis software. Effects on angiogenesis was detected using subsequent immunhistochemical staining for VEGFa. To determine IL-6's cellular origin and its possible local effects on cell proliferation, angiogenesis and immune response, a novel multiplex-fluorescence-in-situ-hybridisation method (miFISH) was used.

Results In comparison to cold storage (n = 20), after reperfusion (n = 30) a significantly increased IL-6 expression could be measured in the BDs ($p < 0.0001$). Patients who did not develop BD complications had received BDs with significantly increased IL-6 amounts and increased VEGFa levels compared to patients who did develop complications (p=.001;.03). IL-6 and VEGFa were co-expressed subepithelial and in peribiliary glands. Fibroblasts (aSMA+) and endothelial cells (CD34+) could be identified as a cellular origin of IL-6, whereas BD epthelial cells (CK19+) and infiltrating leukocytes (CD45+) showed no IL-6 expression.

Conclusions For the very first time it was possible to show an upregulation of IL-6-expression in the BD in-situ following reperfusion. The results suggest that IL-6 indeed prevents BD damage and induces regeneration and angiogenesis of damaged BDs, thus leading to lower probabilities of complications. In the future, the perfusion of IL-6 through the donor's BD might be a therapeutical approach to reduce complication rates after liver transplantation.

2.3 Whole-genome sequencing of carbapenem-resistant gram-negative bacteria in patients with cirrhosis

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Objectives In patients with liver cirrhosis, colonization and infection with carbapenem-resistant gram-negative bacteria (CRGN) are associated with high mortality prior and after liver transplantation. Since the epidemiological context of newly occurring carbapenem resistance (CR) often remains unclear, approaches to identify transmission routes are urgently needed to avoid the spread of CRGN.

Methods Among 351 patients evaluated for liver transplantation and screened for CRGN between 2009 and 2018, 18 CRGN were isolated from 17 patients. In these strains, whole-genome sequencing was performed to assess the genotype and phenotype of CR as well as phylogenetic relationship. Clinical records in patients with closely related strains were dissected to identify potential routes of transmission.

Results Carbapenem-resistant *Klebsiella pneumoniae* (n = 8) and *Pseudomonas aeruginosa* (n = 7) were the predominating pathogens. *In silico* detection of antimicrobial resistance genes for all bacterial strains revealed a diverse spectrum of carbapenemases (VIM-1, VIM-2, OXA-48-like, OXA-24-like and OXA-51-like), each of which was unique to exclusively one of these strains. Core genome phylogenetic analysis for strains of each bacterial species suggested a potential relationship of three *K. pneumoniae* isolates. However, the subsequent in-detail genomic analysis of these strains unveiled differences in several chromosomal loci including a locus involved in colistin resistance. No evidence of plasmid hospitalism conveying CR was detected. From first clinical *de novo* CRGN detection onwards, patients with closely related strains had never simultaneously been admitted to a particular ward, nor had they crossed paths within in-house facilities on the same day.

Conclusion Our data suggest that the phenotypically identified "carbapenem resistance" in our cohort of liver cirrhosis patients was a result of several different underlying genetic mechanisms. In regard to the apparently close relation of three particular isolates, there was no molecular or clinical evidence of in-hospital transmission of CR genes. Since CRGN transmission may occur in very few cases despite strict in-house hygiene measures, comprehensive hygiene concepts including healthcare and nursing providers are warranted.

Lectures Session III Metabolism (incl. NAFLD) Friday, January 29, 2021, 6.20 pm – 7:05 pm, Lecture Hall Virtual Venue

3.1 Identification of hyperammonemia-induced changes in the cerebral transcriptome and proteome

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Question The molecular changes underlying neurological impairment in hyperammonemic disorders such as in hepatic encephalopathy (HE) are only incompletely understood. In the present study we investigated gene and protein alterations in different brain regions of mice with systemic hyperammonemia resulting from knockout of hepatic glutamine synthetase (LGS-KO) and analyzed potential functional consequences in astrocytes *in vitro*.

Methods Proteome and transcriptome analyses were performed by mass spectrometry and gene array, respectively. mRNA and protein levels were analyzed by qPCR, Western blot and immunofluorescence analysis.

Results Using transcriptomics and proteomics we identified 214,44 and 163 mRNA species and 4, 36 and 15 proteins with altered levels in the cerebral cortex, the hippocampus and the cerebellum of LGS-KO mice, respectively. Differentially-expressed genes related to oxidative and endoplasmic reticulum stress, energy metabolism and cell proliferation and others. For selected candidates CARM1, TROVE2, LCN2 and ASCT2 we found, that all of them are expressed by astrocytes in mouse and rat brain as shown by immunofluorescence analyses and that NH_4Cl (5mM, 72h) changed their protein and mRNA levels in rat astrocytes *in vitro* similar to what was found in brains of LGS-KO mice. Functional consequences of the protein level changes of the selected candidates were investigated in cultured rat astrocytes using pharmacological inhibitors of CARM1, ASCT2, iron chelators or siRNA-mediated knockdown of TROVE2. The results suggest a role of CARM1 and ASCT2 for senescence, of LCN2 for disturbed iron homeostasis and of TROVE2 for RNA quality control in NH_4Cl-exposed astrocytes. Challenging rats with ammonia acetate (4.5mmol/kg BW, 24h) also elevated ASCT2 and LCN2 protein levels in cerebral cortex and ASCT2 and TROVE2 mRNA levels were also elevated in *post mortem* brain tissue from patients with liver cirrhosis with HE.

Conclusions In the present study we identified previously unknown changes in the cerebral transcriptome and proteome potentially relevant for hyperammonemia-induced neurological dysfunction in HE. The findings suggest an important role of the methyltransferase CARM1, the glutamine transporter ASCT2, the iron chelator LCN2 and the RNA binding protein TROVE2 for ammonia toxicity and the pathogenesis of HE.

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3.2 Hedgehog Signaling - a mediator between liver and adipose tissue

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DOI 10.1055/s-0040-1721951

Background For many years the understanding and research of Hedgehog (Hh) signaling was limited to embryogenesis and cancer development. However, the functions of Hh in adult organs in control of metabolism and maintenance of homeostasis become more important as Hh is implied in many metabolic disorders such as non-alcoholic fatty liver disease (NAFLD). Active Hh signaling could be detected in mature hepatocytes and is linked to fatty acid metabolism. Previous experiments have also shown morphologic changes in adipose tissue in consequence of Hh inactivation in the liver. The crosstalk of liver and adipose tissue is a complex process for which the role of hepatic Hh signaling has not been investigated yet.

Methods Primary hepatocytes, serum and the different types of adipose tissue – visceral, subcutaneous and brown – were isolated from mice with specific inactivation of Hh signaling in hepatocytes. Morphologic and biochemical characterization of adipose tissue was conducted with immunohistochemistry including analysis of adipocyte size as well as qPCR. A fibroblast growth factor 21 (FGF21) ELISA was performed.

Results Mice with an inactivation of Hh signaling in hepatocytes show a distinct phenotype. Weight of all types of adipose tissue is increased in both sexes. Additionally a changed distribution of cell size can be detected. On the gene expression level, inactivation of Hh signaling was associated with an increased expression of brown and beige adipocyte markers and decrease in the expression of a marker associated with lipogenesis. Immunohistochemistry reveals UCP-1-positive cell clusters in visceral and subcutaneous adipose tissue, indicating a browning effect. As a signal molecule, which is responsible for the observed peripheral communication of the liver with the AT, we found FGF21 as a potential candidate as it is accumulated in the culture supernatant of hepatocytes of transgenic knockout mice compared to the wild type *in vitro*.

Conclusion Hepatocyte-specific inactivation of Hh leads to extrahepatic phenotypic and metabolic changes in the adipose tissue of mice, resulting in the emergence of brown adipocytes in subcutaneous and, more intensely, in visceral adipose tissue. The results reveal a novel and interesting aspect of how morphogenic pathways control metabolism through inter-organ communication. The effects of hepatic Hh could be mediated by FGF21 as a downstream target, which in turn activates adipocyte browning.

3.3 Serum concentrations of classic liver enzymes are unsuitable for diagnosing non-alcoholic steatohepatitis

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DOI 10.1055/s-0040-1721952

Introduction The prevalence of non-alcoholic fatty liver disease (NAFLD) in the German population is 20–30 %. This large number of potentially affected people rules out performing a liver biopsy to confirm a possible diagnosis of non-alcoholic steatohepatitis (NASH) or for the purpose of risk assessment in all cases. Non-invasive methods for diagnosis and to support screening strategies are therefore required. Elevated serum concentrations of classic liver enzymes are currently used as an indicator for NAFLD or NASH.

Goals In this study we aimed to examine whether an adjustment of the upper limit of normal for classic liver enzymes (AST, ALT and GGT) leads to an improvement of the diagnostic accuracy.

Methods Data from 363 morbidly obese patients (42.5 ± 10.3 years old; mean BMI: 52 ± 8.5 kg/m^2) who underwent bariatric surgery, with histologically confirmed NAFL or NASH (NAS and SAF score available), were evaluated using statistical methods.

Results In 121 women (45 %) and 45 men (46 %) we found elevated values for at least one of the three parameters ALT, AST or GGT. The serum concentrations of ALT ($p < 0.0001$), AST ($p < 0.0001$) and GGT ($p = 0.0023$) differed significantly between patients with histologically confirmed NAFL and NASH, regardless of whether the classification was based on the NAS or the SAF score. All three serum parameters correlated significantly positively with the NAS and the SAF score, with correlation coefficients between 0.33 (correlation of ALT with NAS) and 0.40 (correlation of GGT with SAF score). In order to assess whether it is possible to separate NAFL and NASH using classic liver enzymes, ROC curves were generated for all three parameters. The area under the ROC curve achieved a maximum of 0.70 (for ALT applying the NAS), which demonstrates a poor to moderate separation. To achieve 95 % specificity, the upper limit of normal for ALT would have to be 47.5 U/l; for 95 % sensitivity, the upper limit of normal for ALT would have to be 17.5 U/l, which leads to a diagnostic gap of 62 % uncategorized patients.

Discussion ALT, AST and GGT are unsuitable for the non-invasive diagnosis of NAFL or NASH. Furthermore they are not applicable as a screening tool for NASH in obese patients. The suitability of classic liver values as an indicator for NAFLD should generally be questioned.

Poster Visit Session I Basic Hepatology (Fibrogenesis, NPC, Transport) Friday, January 29, 2021, 12:30 pm – 1:15 pm, Poster Session Virtual Venue

1.4 JNK mediates differential protection in hepatocytes and non-parenchymal cells during cholestasis

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Background Chronic cholestasis triggers severe liver injury, inflammation and subsequently liver fibrosis associated with c-Jun N-terminal kinases (JNK) activation. However, the JNK specific role during cholestasis in parenchymal and non-parenchymal cells (NPCs) has not been defined yet. Previously, we demonstrated that *Jnk1* in hepatocytes has no impact on chronic hepatic injury during experimental fibrosis. Here, we investigated the relevance of *Jnk2* but also *Jnk1* and *Jnk2* specifically in hepatocytes and NPCs for cholestatic liver fibrosis.

Methods JNK activation was investigated in patients with cholestatic liver disease namely primary biliary cholangitis (PBC) and primary sclerosing cholangitis (PSC) (n = 27). Wildtype (WT), hepatocyte-specific knockout mice for *Jnk2* ($Jnk2^{\Delta hepa}$) or *Jnk1* and *Jnk2* ($Jnk1^{\Delta hepa}/2^{\Delta hepa}$) were generated. Additionally, *Jnk2* knockout ($Jnk2^{-/-}$), $Jnk1^{\Delta hepa}/2^{-/-}$ and *Mdr2* knockout mice were included. Mice were subjected to bile duct ligation (BDL) for 28 days. Hepatic damage, cell death, proliferation, inflammation and fibrosis were assessed by immunostainings, Western Blot and qRT-PCR. Additionally, microarray analysis and bone marrow transplantation (BMT) were performed.

Results Protein expression of pJNK was significantly increased in human PSC and PBC samples and correlated with disease activity. Furthermore, mice with surgically- or genetically-induced cholestasis (BDL and $Mdr2^{-/-}$) showed increased pJNK expression. After BDL, $Jnk2^{\Delta hepa}$ livers showed no significant differences in hepatic injury, inflammation and fibrosis compared to WT controls. In contrast, $Jnk1^{\Delta hepa}/2^{\Delta hepa}$ livers exhibited exacerbated liver damage and immunostainings of liver sections revealed increased cell death, proliferation, inflammation and fibrogenesis demonstrating a combined protective role of *Jnk* genes in hepatocytes during cholestasis. Moreover, $Jnk2^{-/-}$ and $Jnk1^{\Delta hepa}/2^{-/-}$ mice displayed increased liver injury and fibrosis compared to $Jnk2^{\Delta hepa}$ and $Jnk1^{\Delta hepa}/2^{\Delta hepa}$ mice, respectively. Finally, BMT experiments demonstrated the involvement of *Jnk2* in NPCs but not in BM-derived cells in controlling BDL-dependent disease progression.

Conclusion Combined but not individual function of *Jnk1* and *Jnk2* in hepatocytes is essential to protect against BDL-induced liver fibrosis. *Jnk2* in NPCs but not BM-derived cells confers additional protection during cholestasis. Hence JNKs have a differential and cell-type specific protective role during cholestasis.

1.5 A so far unrecognized mechanism of acetaminophen hepatotoxicity

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Acetaminophen (APAP) is one of the most frequently used antipyretic and analgesic drugs. Overdoses lead to hepatotoxicity due to metabolic activation by the CYP450 enzymes, mainly CYP2E1, and excessive formation of the toxic intermediate N-acetyl-p-benzoquinone imine. Despite the extensive knowledge of the mechanism of APAP hepatotoxicity, the only approved therapy is the administration of N-acetylcysteine, which is limited by a narrow time window. In this study, the role of bile acids in APAP-induced liver injury was investigated. For this purpose, a mouse model of APAP-induced hepatotoxicity was used. Alterations in bile acid transport and canalicular morphology were evaluated by intravital two-photon imaging. Key findings were followed up by matrix-assisted laser desorption ionization (MALDI MSI) imaging, clinical chemistry, histopathology, and immunohistochemistry. The results revealed that early after APAP intoxication, a breach of the bile-blood barrier occurred in the pericentral compartment of the liver lobule, which led to recirculation of bile acids between bile canaliculi, sinusoids, and hepatocytes. This exposed the pericentral hepatocytes to very high concentrations of bile acids. Interestingly, this transient stage of cholestasis occurred before the increase of transaminases activity in the blood, which excludes that this increase in bile acid concentrations is because of liver dysfunction following hepatocyte death. In order to study if the observed transient cholestasis is a key event relevant for the subsequent hepatocyte death, we performed an experiment to block the hepatocyte uptake of bile acids from the sinusoidal blood. For this purpose, the NTCP inhibitor Myrcludex B (5 mg/kg, i.v.) was administered to Oatp knockout mice; simultaneously, the mice received a toxic dose of APAP (300 mg/kg), and then the liver damage was evaluated 24 hours later. Interestingly, blocking the sinusoidal uptake of bile acids strongly reduce APAP hepatotoxicity in comparison to the wild-type mice. In conclusion, the results demonstrate a transient stage of cholestasis which is a key event in the pathogenesis of APAP-induced hepatotoxicity. Therapeutic exploitation of this mechanism by blocking the sinusoidal uptake transporters of bile acids strongly ameliorated APAP-induced liver injury.

1.6 HNF4α and FOXA2 form a hierarchical regulatory network to guarantee Albumin synthesis in response to pathophysiological challenge

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Background and Aims Albumin is indispensable for homeostasis. In healthy humans, only hepatocytes synthesize albumin. To date, it is largely unknown how the liver produces albumin in physiological and diseased microenvironments. In this study, we scrutinize a hierarchical transcriptional regulatory network that controls albumin expression in response to different physiological and pathophysiological challenges.

Methods Albumin levels in liver tissues and serum were examined in 157 patients with end-stage liver diseases, including 84 hepatocellular carcinoma, 38 decompensated cirrhosis and 35 acute on chronic liver failure (ACLF). Candidate regulatory transcription factors HNF4aand FOXA2 were measured by IHC in these patients. The mechanism how HNF4a and FOXA2 regulated albumin transcription and expression was investigated in human and mouse primary hepatocytes and liver progenitor cell (LPC) lines HepaRG and BMOL.

Results Most patients with end stage liver disease maintain serum albumin concentrations in the normal range. In collected liver tissues, immunohistochemistry shows albumin expression in hepatocytes. However, in a subcohort of patients with massive hepatocyte loss, albumin is expressed in activated LPC. *In vitro*, ChIP assays reveal that HNF4a and FOXA2 are capable of binding to the albumin promoter. Knockdown of either of them by RNAi reduces albumin expression in both cell types. Immunohistochemistry analyses further show that in normal and non-cirrhotic livers, HNF4a, a constitutively expressed lineage transcription factor, but not FOXA2, is robustly expressed in hepatocytes. However, in a large portion of cirrhotic livers, HNF4a expression is undetectable by immunohistochemistry. In these patients, FOXA2 is remarkably expressed in hepatocytes. Further, FOXA2 positive staining is closely associated with Sonic hedgehog (SHH) induced Gli2 expression. *In vitro*, disruption of Sonic hedgehog signaling influences FOXA2 expression in hepatocytes. In patients with massive hepatocyte loss, active LPCs not only express albumin, but also display HNF4a or FOXA2, indicating that the two transcription factors are required for Albumin expression in LPCs.

Conclusions HNF4a and FOXA2 form a hierarchical regulatory network to guarantee essential albumin expression in response to different physiological and pathophysiological challenges.

1.7 Fibroblast growth factor 21 response in a preclinical alcohol model of acute-on-chronic liver injury

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Background Fibroblast growth factor (FGF) 21 has recently been shown to play a potential role in bile acid metabolism. Our aim was to investigate the FGF21 response in the ethanol-induced acute-on-chronic liver injury (ACLI) model in *Abcb4*-/- (KO) mice with deficiency of the hepatobiliary phosphatidylcholine transporter.

Patients and methods Total RNA was extracted from *Abcb4*-/- mice and wild-type (WT, C57BL/6J) controls, which were either fed control diet (WT/Cont and KO/Cont groups; n= 22/group) or ethanol diet, followed by an acute ethanol binge (WT/EtOH and KO/EtOH groups; n= 22/group). A total of 58 human subjects were recruited into the study including alcohol-associated liver disease patients (AALD; n = 31) and healthy controls (n = 27). Hepatic and ileal expression of genes involved in bile acid mechanism, plasma FGF concentrations (ELISA) and bile acid levels were analyzed. Serum concentrations of human oxysterols 7α and 27-hydroxycholesterol were quantified by isotope dilution GC-mass spectrometry selected ion-monitoring (GC-MS-SIM). Primary mouse hepatocytes were used for cell culture experiments.

Result Alcohol feeding significantly induced FGF21 and decreased hepatic *Cyp7a1* levels. Hepatic steady-state mRNA levels of *Fxr, Shp, Fgfr1* and *Fgfr4*, and plasma FGF15/FGF19 levels did not differ with or without alcohol challenge. Recombinant human FGF21 (rhFGF21) suppressed *Cyp7a1* in a dose-dependent manner *in vitro*. AALD patients showed markedly higher FGF21 and lower 7α-OHC plasma levels in the setting of constant FGF19 concentrations.

Conclusions Simultaneous upregulation of FGF21 and repression of *Cyp7a1* expression upon chronic plus binge alcohol feeding together with invariant plasma FGF15 and hepatic *Shp* and *Fxr* expression suggest the presence of a direct regulatory mechanism of FGF21 on bile acid homeostasis through inhibition of CYP7A1 by an FGF15-independent pathway in the ACLI model. In accordance with the *in vivo* results, our translational human studies demonstrate significant FGF21 upregulation and significant reduction of serum 7αOHC levels in AALD patients upon ongoing ethanol consumption with unchanged plasma FGF19 levels. The *in vitro* studies indicate a direct effect of FGF21 on hepatic *Cyp7a1* mRNA suppression in a dose-dependent manner.

1.8 Expression of paracrine fibroblast growth factors in hepatic stellate cells and hepatic fibrosis

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Fibrosis is characterized by accumulation of extracellular matrix and is highly conserved following tissue injury. The regulation of fibroblast growth factor (FGF) signaling is a prerequisite for adequate wound healing in various organs. The FGF family contains 22 proteins that are divided in seven subfamilies and can be classified into paracrine, intracrine and endocrine factors. FGFs signal through tyrosine kinase FGF receptors (FGFRs). Hepatic fibrosis represents a chronic wound healing response and hepatocellular carcinoma (HCC) frequently develops in this “non-healing” wound. Although FGFRs are targets for the treatment of HCC, the expression of FGFs in hepatic fibrosis is still poorly understood.

The aim of this study was to analyze the expression of paracrine FGFs in HSC and hepatic fibrosis.

Methods and Results RT-qPCR revealed that primary murine and human HSC express following paracrine FGFs: (i) FGF1-subfamily: FGF-1/2, (ii) FGF4-subfamily: FGF-5, (iii) FGF7-subfamily: FGF-7/10/22, (iv) FGF8-subfamily: FGF-17/18 and (v) FGF9-subfamily: FGF-9/16. During the course of *in vitro* HSC activation expression of following FGFs significantly increased: (i) FGF-1/2, (ii) FGF-5, (iii) FGF-7/10/22, (iv) FGF-18 and (v) FGF-9. Fitting to this, these FGFs were also elevated in murine models of hepatic fibrosis (bile-duct-ligation, thioacetamide-induced toxic liver injury and diet-induced models of non-alcoholic steatohepatitis). To enhance the dependability of our data, we analyzed the correlations between the FGFs and markers of hepatic stellate cell activation, COL1A1 and ACTA2 applying GEPIA database and GTEx dataset of human liver tissues. (i) FGF-1/2, (iii) FGF-7/10, (iv) FGF-18 and (v) FGF-9 significantly correlated with COL1A1 and ACTA2 indicating activated HSC as cellular source of FGFs. RT-qPCR analysis in activated HSC from human donors showed pronounced variations in FGF expression pattern indicating there are general high and low “FGF-expressors”.

Summary and conclusion Several paracrine FGFs are significantly upregulated during HSC activation and in experimental models of hepatic fibrosis. Also in human fibrotic liver tissues, HSC appear as major FGF-source. The observed donor-to-donor variation in FGF expression in activated HSC might play a role in the variability of the clinical course of liver fibrosis. Further studies are needed to elucidate the potential of defined FGFs as biomarkers or therapeutic targets in patients with chronic liver disease.

1.9 Targeted deletion of Tgr5 in the intestine leads to less biliary damage in cholestasis

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Introduction Tgr5 (Gpbar1) is a G protein coupled receptor responsive to bile acids (BA) which is expressed in cholangiocytes and the epithelium of the small intestine (1). We have previously demonstrated that mice with a targeted deletion of Tgr5 are more susceptible towards cholestatic liver injury (2,3). Aim of this study was to investigate the role of Tgr5 in the intestine during cholestasis, which was induced by a 1 % lithocholic acid (LCA) diet for 84h (3) using the Tgr5 Villin Cre knockout mouse model.

Methods 8-12 week old Tgr5 Villin Cre knockout ($Tgr5^{Vil\text{-}Cre\text{-}/\text{-}}$) and wildtype (Wt) mice were fed a 1 % LCA diet for 84h or standard chow diet. After 84h serum, as well as liver and intestinal tissue were collected. Serum markers of liver and biliary damage were determined using Spotchem-biochemical analyzer. Tgr5, Asbt, Fgf15, Col1a1, Col1a2 and α-SMA mRNA and protein expression was analyzed by real-time PCR and western blotting.

Results Both genotypes showed severe liver damage in response to LCA feeding, which was reflected by high serum levels of aspartate (AST) and alanine aminotransferase (ALT). However, $Tgr5^{Vil\text{-}Cre\text{-}/\text{-}}$ were less susceptible towards LCA-induced biliary damage as demonstrated by a significantly lower increase of alkaline phosphatase (ALP) and bilirubin levels as well as by histopathology of extrahepatic bile ducts. $Tgr5^{Vil\text{-}Cre\text{-}/\text{-}}$ mice had lower expression of fibrosis markers (Col1a1, Col1a2 and α-SMA) after 84h of BS-enriched diet. Tgr5 mRNA and protein expression were significantly suppressed in the small and large intestine, while Tgr5 mRNA and protein expression in the hepatobiliary system was not altered in comparison to Wt levels. In the ileum, a significant reduction of Asbt mRNA expression and higher Asbt protein levels were observed together with a significant increase of Fgf15 mRNA in $Tgr5^{Vil\text{-}Cre\text{-}/\text{-}}$ mice.

Conclusion Deletion of TGR5 in the intestinal epithelium reduced LCA induced biliary damage. Mechanisms contributing are upregulation of Fgf15 and retained TGR5 expression in cholangiocytes.

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1.10 Effects of SNPs in HE-relevant genes for the development of hepatic encephalopathy

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Background Hepatic encephalopathy (HE) is a frequent complication in acute and chronic liver failure and a negative prognostic factor for mortality in patients with liver cirrhosis. Currently, HE is seen as the clinical manifestation of a low grade cerebral edema and cerebral oxidative stress which disturbs oscillatory networks and synaptic plasticity in brain.

Aims and Methods To identify genetic risk factors for the development of a hepatic encephalopathy a Next Generation Sequencing (NGS) chip was developed, representing the whole coding region including selected promoter regions in 7 genes that potentially play a role in the pathogenesis of HE. DNA samples were collected from 48 patients with liver cirrhosis and at least one episode of hepatic encephalopathy, 34 patients with diagnosed liver cirrhosis who never had an episode of HE so far and 30 controls, with no known liver disease. In addition DNA samples were collected from frozen brain tissue from donors of the body donor program of the University of Düsseldorf (9 patients with cirrhosis and HE, 7 controls without liver cirrhosis).

Results 55 SNPs could be identified which were not specific for patients with liver cirrhosis and hepatic encephalopathy and were also found in controls and patients with liver cirrhosis without an episode of HE. For the glutamate transporter 1 (GLT-1) we could identify two SNPs (rs752949, rs1042113) that have a lower allele frequency in patients with liver cirrhosis and HE than in the control group of patients with no liver cirrhosis or our additional control group from the database gnomAD without reaching statistically significance yet. This was also the case for the SNPs rs3027958 and rs1042113 of SCL1A5.
Of particular interest we could identify 2 SNPs of SLC1A5 (rs1060043 and rs2070246) with a statistically significant increase of their allele frequency in patients with liver cirrhosis and HE compared to the group of patients with liver cirrhosis and no known episode of HE (18 % vs. 3 % and 27 % vs. 11 %).

Discussion Our study suggests that SNPs may play a role for the pathogenesis of HE. However, further patients need to be included in this study to validate the present findings and further research is needed to identify consequences of the individual SNPs for the respective protein function.

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1.11 Identification of miRNA148a-3p as a novel non-invasive potential biomarker for bacterial infection-related ACLF

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Background miRNAs hold great promise as potential *biomarkers in* liver diseases including acute-on-chronic liver failure (ACLF). Bacterial infection (BI) is a common precipitant of ACLF in humans. Herein, we **aim** to identify putative miRNA biomarkers in a novel bacterial infection-related acute-on-chronic liver injury model (BI-ACLI), mimicking ALCF conditions with LPS injection in a knock-out mouse with chronic hepatobiliary injury (*Abcb4*-/-).

Methods Fifteen-week-old C57BL/6J (wild-type) or *Abcb4*-/- (knock-out) mice (N = 16 each) were treated with IP injections of either LPS (4 mg/kg) or sterile saline solution (0.9 % NaCl). Transcriptomics were characterized by RNA sequencing (RNA-Seq) of mRNA libraries constructed from liver samples of three randomly selected male mice from each group (NebNext Ultra II RNA library preparation kit) using NextSeq500 (Illumina). Liver-specific steady-state mRNA levels (relative to *Gapdh*) of several differentially expressed genes (DEGs) identified by RNA-seq were confirmed by Taqman assays (Applied Biosystems). Screening of differentially expressed miRNAs was performed in the same samples by miScript miRNA PCR arrays (Qiagen). For mouse-to-human translation, identified miRNAs in mouse livers were further quantified in plasma samples of healthy controls and patients with chronic liver diseases (CLD) with or without bacterial infection.

Results Trabscriptomic data revealed 145 DEGs, which were significantly up- (N = 127) or down-regulated (N = 18) in KO-LPS group compared to their counterparts. Differential expressions of identified DEGs were found to be in line with RT-PCR results for a number of genes tested, including *Rantes, IL-22, IL-2* and *IL-6.* Seven out of 84 miRNAs screened were shown to be differentially expressed in ACLI mice (KO-LPS), namely let-7c-5p, let7b-5p, mir148a-3p, mir21a-5p, mir23a-3p, mir25-3p, and mir29c-3p. Of these seven miRNAs,

mir148a-3p was further confirmed to be significantly elevated in CLD patients with current BI (time of BI detection < 2 weeks, N = 14), compared to those without BI (p=0.038, N = 15), with past BI (time of BI detection > 1 year, N = 16) (p=0.043) and healthy controls (p < 0.01, N = 7).

Conclusions Differential expression of hepatic cytokines, chemokines and miRNAs in *Abcb4*-/- mice upon LPS challenge provide hints for identifying potential biomarkers in this dual-hit model. Our *in vivo* and human findings suggest that circulating mir148a-3p might be a potential marker for BI-related ACLF.

1.12 Liver progenitor cells regulate ductular reaction and induce fibrosis upon severe liver injury via RAGE signaling

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The origin and role of liver progenitor cells (LPCs) also known as atypical biliary epithelial-like cells have been a controversial topic from their involvement in regeneration during liver damage to being associated with severe liver disease progression, fibrosis and tumor initiation. Enhanced proliferation of LPCs, called the ductular reaction, leads to portal fibrogenesis - characterized by stellate cell activation, ECM deposition, inflammation. The receptor for advanced glycation end products (RAGE) signaling axis is often associated with this chronic inflammation-associated tissue damage and plays an essential role in modulating the tumor microenvironment. RAGE mediates the LPC expansion, onset of liver fibrosis and HCC formation. LPC expansion, commonly manifests as a ***"Ductular reaction (DR)"*** in chronic liver disorder.

To investigate if RAGE also drives the ductular reaction in cholestatic (bile acid) mediated liver injury, we investigated CDE-diet induced ductular reactions in a mouse model with LPC-specific RAGE knockout and labelled LPCs. Ablation of RAGE in LPCs strongly impaired ductular reaction. Strikingly, this was accompanied in vivo by reduction of activated hepatic stellate cells that populate in the liver as response to fibrosis. *In vitro* transcriptomic studies of isolated primary LPCs stimulated with supernatants from necrotic hepatocytes showed that stress response, inflammatory and pro-fibrotic pathways were enriched in LPCs upon treatment with necrotic medium. Most interestingly, signaling pathways that regulate organ size, tissue homeostasis and cell survival pathways were found to be RAGE-dependent. Clusters of stem cell renewal-related genes were deregulated upon ablation of RAGE. In line with the whole transcriptome profile, we demonstrated that ablation of RAGE attenuates LPCs organoid-forming ability, implying that RAGE regulates stemness properties of LPCs.

Our recent results demonstrated that RAGE is required for LPCs activation and proliferation, as well as the crosstalk with stellate cells in supporting fibrogenesis. Taken together, our data uncover a potential mechanistic insight on the role of RAGE in LPCs in association with fibrosis upon chronic liver injury.

Keywords: ECM-Extracellular matrix, CDE- Choline-deficient-ethionine

1.13 *Schistosoma mansoni* infection induces DNA damage in hamster liver

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Question *Schistosoma mansoni* infection induces oxidative stress in the liver, which so far presumably is caused by cells of the immune system. During co-infections with hepatitis B and C virus there is an increased risk for cancer development. SEA (Soluble Egg Antigen) isolated from *S. mansoni* eggs, activates carcinogenesis associated JNK/STAT3 pathways. With *in vivo* and *in vitro* studies, we aimed to analyze whether *S. mansoni*-induced oxidative stress is able to induce DNA damage and thus increase the risk for mutagenesis.

Methods Hamster livers of three different experimental groups (not infected, or infected with a single or both genders of *S. mansoni*) were used for western blotting, malondialdehyde (MDA) assay and catalase activity analysis. In cell culture experiments with HepG2 cells, we used SEA (Soluble Egg Antigen) to stimulate the cells as well as reduced glutathione (GSH) to prevent oxidative stress. Analyses have been performed by western blot, MDA assay and Comet assay.

Results In HepG2 cells SEA induced DNA damage. We show that GSH prevents oxidative stress and thus normalizes SEA-induced DNA damage. Comparing the oxidative stress levels, SEA-treated HepG2 cells and *S. mansoni*-infected hamsters show higher levels of the oxidative stress marker MDA than the control groups. The DNA repair mechanism protein p-H2AX is activated in SEA-stimulated cells and *S. mansoni*-infected hamsters. SEA-induced activation of JNK/STAT3 pathways and p-H2AX are normalized in cell culture experiments by simultaneous GSH-treatment.

Conclusions *S. mansoni* induces hepatic oxidative stress *in vivo* and *in vitro*. Oxidative damage and consequently DNA damage in hepatocytes is caused by *S. mansoni* even during a lack of cells of the immune system. This implicates that the oxidative stress is not only caused by cells of the immune system, but additionally SEA itself causes hepatic oxidative stress. GSH is able to prevent SEA-treated cells from DNA damage and carcinogenesis-associated pathway activation.

1.14 Parasite eggs induce metabolic stress in *Schistosoma mansoni*-infected hamster livers

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Question Schistosomiasis is one of the most common parasitic diseases, affecting approximately 240 million people worldwide. During egg deposition, many of the parasite eggs are swept away in the host circulation via the mesenteric vessels and become trapped in the liver. Subsequently, granuloma formation and a fibrotic-inflammatory remodelling are induced. Results of previous studies demonstrated that an infection with *Schistosoma mansoni* influences the hepatic carbohydrate metabolism. The aim of the present study was the characterization and mechanistic analysis of the alterations in the hepatic carbohydrate metabolism induced by secreted factors of *S. mansoni* eggs.

Methods The expression of key enzymes of the hepatic carbohydrate metabolism was analyzed by western blot and immunohistochemistry in liver lysates of *S. mansoni* bisex-infected hamsters (n = 5, age 4.5 months, 7 weeks after infection). Singlesex-infected hamsters (n = 5) and non-infected hamsters (n = 3) served as controls. The hepatic glycogen content was measured by a quantitative assay. In addition, enzymatic and signaling pathway-associated

relationships were mechanistically demonstrated by stimulation and inhibition experiments.

Results In comparison to non-infected and singlesex-infected control animals, the key enzymes of glycolysis were induced in *S. mansoni* bisex infected hamster livers, while gluconeogenesis was not affected. In addition, the key enzymes of glycogen metabolism as well as hepatic glycogen were decreased to 20 % of the normal value of healthy controls. In contrast, glycogen content appeared enriched in the parasitic eggs, whereas an exhaustive depletion of glycogen in the parenchyma was demonstrated by periodic acid-Schiff (PAS)-staining. The results from the hamster model were supported by data of *in vitro* experiments, which aimed at stimulating HepG2 cells with soluble egg antigen (SEA) and inhibiting central metabolic signaling pathways (Akt, AMPK).

Conclusions The increased hepatic energy supply is probably important for the maintenance of the vital functions of the eggs, including embryogenesis. These results of the hamster model might explain the extended survival (months) of hepatic eggs from *S. mansoni* in comparison to the short survival period after excretion (days). In addition, the parasite appears to control these processes actively as secreted factors of the eggs activate the relevant metabolic pathways in cell culture.

1.15 During liver regeneration active TGFb drives polarization of distinct CCR2-dependently recruited macrophage populations

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Background & aims In the context of liver regeneration macrophages play an important regulatory role. Thereby the changes of the composition of the different macrophage populations and of their polarization during liver regeneration has not been investigated in detail. Likewise, the factors that mainly control this process as well as their relevance for undisturbed regeneration are unclear.

Methods Wild type animals, CCR2-/- mice and mice with myeloid cell specific deletion of the TGFβRII were analysed after partial hepatectomy (PHx). In addition, the impact of hepatocytes on macrophage polarization was studied using a coculture with highly purified hepatocytes which are embedded into a collagen matrix.

Results After PHx there is an CCR2-dependent increase in macrophages characterized by high expression of CD11b (F4/80+/CD11bhigh), while the number of low CD11b expressing macrophages (F4/80+/CD11blow) decreases significantly and only gradually increases again during the regeneration process. Thereby, CD11bhigh macrophages can be clearly further distinguished into two subgroups based on their CD14 expression and those characterized by high expression of CD11b and of CD14 show a temporary but early, rapid and strong increase during regeneration after PHx. Notably, these macrophages exhibit a particular polarization under homoeostatic conditions and, compared to the other macrophage populations of the liver, also undergo significant changes of their polarization in the course of the regeneration process. Moreover, their presence in the liver as well as their recruitment is CCR2 dependent, which is not the case for F4/80+/CD11blow/CD14low macrophages and only in part for those which are F4/80+/CD11bhigh/CD14low. The experiments further suggest that the availability of active TGFβ plays a role in the intercellular communication network by which hepatocytes influence the polarization of in particular F4/80+/CD11bhigh/CD14high macrophages and lack of the TGFβ receptor II in macrophages results in prolongation of the proliferation phase of hepatocytes, accelerated regeneration as well as reduced injury after PHx.

Conclusion Recruitment of macrophages into the regenerating liver is CCR2-dependent. Different macrophage populations are recruited into the regenerating liver, which differ substantially in their willingness to adapt their polarization. In this context, the availability of active TGFβ seems to play a role and influence the regeneration process.

1.16 Liver cell swelling leads to upregulation of miR-141-3p in perfused rat liver and primary rat hepatocytes

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Introduction Small persistent changes in liver cell hydration can occur in response to ambient osmolarity, hormones, cumulative amino acid uptake or oxidative stress. Alterations of cell hydration critically contribute to changes in cell functions as well as gene expression. Cell swelling is considered as an anabolic trigger, leads to an inhibition of proteolysis, choleresis, proliferation and is obligatory during liver cell regeneration. On the other hand, cell shrinkage is a catabolic signal and can sensitize towards apoptosis through activation of the CD95 system. In this study we investigated whether miRNAs play a role in hypoosmolarity-induced liver cell swelling.

Methods Rat livers were perfused with either normo- or hypoosmotic fluid in a non-recirculating system over a time course of 180 min. For cell culture experiments hepatocytes were isolated from rat livers by a collagenase perfusion technique. Cultured primary rat hepatocytes were treated with normo- or hypoosmotic medium over a time course of 24 h. Microarray analysis was conducted to screen microRNAs that are differentially expressed in rat livers, which were hypo- (225 mosm/L) and normoosmotically (305 mosm/L) perfused for 180 min. Putative target mRNAs were analyzed via qPCR after 180 min of either hypo- or normoosmotic rat liver perfusion. In rat liver perfusion specific inhibitors for Erk (PD098059; 500 nM), p38 MAPK (PD169316; 250 nM) and microtubules (colchicine, 500 nM) were used.

Results A total of 728 microRNAs were identified, from which 70 were up- and 63 were downregulated in response to hypoosmotic exposure. Specifically, hypoosmotic exposure led to a significant upregulation of miR-141-3p in perfused rat liver as well as primary rat hepatocytes. It was also identified that several putative target mRNAs of miR-141-3p were downregulated after 180 min of hypoosmotic exposure, including Slc39a10 and Dstyk. After addition of PD098059, PD169316 and colchicine, hypoosmolarity-induced upregulation of miR-141-3p was inhibited.

Conclusion Our data indicate that miR-141-3p upregulation is responsive to hypoosmotic stress and that it might contribute to proliferative effects of hypoosmolarity-induced hepatic cell swelling controlled by its target genes. It is shown that both Erk and p38 MAPK play a key role in the hypoosmotic upregulation of miR-141-3p.

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1.17 Bile salt-induced pro-fibrogenic proliferation of hepatic stellate cells is partially mediated by PI3K p110a signalling

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Question Chronic cholestatic liver diseases frequently progress to liver fibrosis. To date, therapeutic options remain scarce. The hydrophobic bile salt Chenodeoxycholic acid (CDCA), accumulating in cholestasis, has been previously shown to stimulate proliferation of hepatic stellate cells (HSCs) and we recently characterised the pro-fibrogenic effects of such signalling (Cells 2020;9 (2):281). Here, we test the role of Phosphatidyl-inositol-3-kinase dependent signalling on bile salt induced activation of HSCs, since this signalling cascade had been implicated in other causes of HSC activation.

Methods HSCs were derived from female FVB/N mice. Expression of catalytic PI3K subunits and activation of this signalling cascade were confirmed by

Western Blotting. HSCs were incubated with CDCA (100 µM) in presence or absence of the PI3K isoform p110a specific inhibitor Alpelisib (up to 5 µM) for up to 14 days. DNA quantification was performed as a surrogate of cell number. *In vitro* collagen deposition was quantified photometrically. The human hepatic stellate cell line LX-2 was activated by TGF-b (10 ng/ml) in presence or absence of Alpelisib (0.5 – 25 µM). PI3K p110a protein expression in LX-2 cells was suppressed by siRNA, followed by 24 h incubation with TGF-b. LX-2 cell activation was evaluated by aSMA quantification.

Results PI3K p110a was found to be expressed in both human and murine HSCs as well as in LX-2 cells. An increase in PKB phosphorylation upon stimulation with CDCA indicated engagement of this signalling cascade. Following incubation with the PI3K inhibitor specific for p110a, Alpelisib, PKB phosphorylation and CDCA-induced increase in DNA amount were diminished. Furthermore, CDCA-induced collagen deposition decreased. Activation of LX-2 cells by TGF-b was reduced by Alpelisib, too. PI3K p110a protein expression was reduced after treatment with siRNA. Lack of p110a prevented TGF-b-induced activation of LX-2 cells.

Conclusion Our results indicate that the pro-fibrogenic properties of CDCA in murine HSCs may partially be mediated by PI3K p110a. In line with this observation, pro-fibrotic signalling in human LX-2 cells seems to depend on this signalling cascade, too. These results warrant further investigation of this signalling pathway in *in vivo* models of cholestasis and liver fibrosis.

1.18 The receptor tyrosine kinase inhibitor Crenolanib improves recovery from liver fibrosis in thioacetamide-treated rats

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Platelet-derived growth factor receptor (PDGFR)-mediated signaling induces proliferation of stellate cells, which contributes to fibrogenesis in chronic liver diseases. Since PDGF levels increase in the blood of patients with fibrotic liver, we addressed the question of whether interfering with PDGFR signaling improves recovery from this disease. To induce fibrous scars in liver tissue, rats were treated with thioacetamide (TAA) for 18 weeks via the drinking water. After cessation of TAA administration, the animals received Crenolanib, which is a benzamidazole derivate capable to inhibit receptor tyrosine kinases (RTK) of class III such as PDGFR-α/β, FMS related tyrosine kinase-3 (FLT3), and c-KIT. Administration of 0.05 mg/ml Crenolanib via the drinking water for 2 weeks significantly improved the recovery of the liver from TAA-induced fibrosis. Crenolanib-treated rats showed a clear regression of nodular appearance, macroscopically visible in fibrotic livers. Moreover, histological analyses of liver sections from these rats showed a significant decrease of Sirius Red staining and of collagen type IV, α-smooth muscle actin, PDGFR-β, and cytokeratin 19 immunofluorescence. Reestablishment of liver zonation, which disappeared during fibrosis, has also been observed in the presence of Crenolanib since glutamine synthetase-expressing hepatocytes reappeared close to blood vessels associated with scar residues. To elucidate the effects exerted by Crenolanib on the fibrotic liver, isolated hepatic stellate cells were treated with 0.1 and 1 µM Crenolanib for 14 days. Crenolanib not only lowered proliferation but also initiated hepatic endoderm specification of stellate cells in a p38 mitogen-activated protein kinase and c-Jun-activated kinase-dependent manner. However, this developmental process remained incomplete, and the stellate cells accumulated lipids, which might be caused by stress-responsive signaling pathways. Inhibition of cell proliferation and activation of a stress response that initiated developmental processes in stellate cells provide explanations for the improved recovery of Crenolanib-treated rats from liver fibrosis.

1.19 Ammonia induces rapid clustering and membrane translocation of GLAST in cultured rat astrocytes

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Background Glutamate homeostasis is disturbed in hepatic encephalopathy (HE) and is thought to play a role in its pathogenesis (1). In cultured rat astrocytes, glutamate is released as a consequence of exposure to ammonia in a calcium-dependent mechanism (2). We observed that short time incubation of cultured rat astrocytes transfected with eYFP-GLAST with ammonia (5mmol/l) induced the formation of GLAST clusters.

Aim Our study aims to improve the understanding of the alteration of glutamate homeostasis in HE by investigating GLAST clustering, membrane translocation and multimerization.

Methods Astrocytes, isolated from cerebral cortex of Wistar rats, were transfected with an eYFP tagged GLAST or/and a mCherry tagged GLAST. Using living cells, Total Internal Reflection Microscopy (TIRFM) was performed to investigate the alteration of localization of eYFP-GLAST-molecules upon addition of ammonia. Multimerization of GLAST was investigated using Fluorescence Resonance Energy Transfer-Fluorescence Life Time microscopy (FRET-FLIM) by looking at variations of fluorescence lifetime and fluorescence anisotropy of eYFP-GLAST/GLAST-eYFP before and after exposition of cultured rat astrocytes to ammonia.

Results Using TIRF microscopy, a statistically significant increase of eYFP-GLAST/GLAST-eYFP clusters was observed at the cell surface after 30 seconds stimulation of cultured rat astrocytes with ammonia (5mmol/l) (n = 4). Furthermore preliminary data from FRET-FLIM microscopy experiments indicated a decrease in fluorescence lifetime and an increase in anisotropy of eYFP-GLAST/GLAST-eYFP in cells expressing eYFP-GLAST/mCherry-GLAST and GLAST- eYFP/GLAST-mCherry upon exposure to ammonia.

Conclusion TIRF microscopy results suggest a translocation of eYFP-GLAST/GLAST-eYFP from the cytoplasm to the cell membrane during GLAST clustering and first FRET-FLIM data indicate a GLAST multimerization upon exposure of cultured rat astrocytes to ammonia.

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- (2) Görg B et al. (2010) Ammonia triggers exocytotic release of L-glutamate from cultured rat astrocytes. Glia. 58:691-705

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1.20 Abcb4*KO* mice upon chemical intoxication represent features of acute-on-chronic liver failure in patients

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Acute-on-chronic liver failure (ACLF) is a major complication in patients with chronic liver diseases. To better understand the pathophysiology and dynamics of ACLF, we developed a mouse model based on a two-hit hypothesis. The first hit is the deletion of *Abcb4* in Balb/c mice, leading to liver fibrosis and ductular

reaction similar to chronic hepatobiliary injury in patients. A sublethal dose (second hit) of CCl4 to 65 week-old Abcb4*KO* mice recapitulates an acute event in ACLF patients, similar to drug intoxication, binge drinking, or trauma. Livers and blood were collected at time points 0, 1, 2 and 4 d after the CCl4 injection. Histologically, massive hepatic necrosis is recorded at day 1 and 2 in mice with good prognosis after CCl4 treatment, whereas almost no necrosis is present in mice with poor prognosis at day 1 after CCl4 injection. A slight increase in Tunel positive cells (reflecting apoptosis) correlates with poor prognosis of mice. Better prognosis is additionally associated with less extrahepatic injury, i.e. kidney injury as indicated by a significant increase of serum creatinine concentrations. ACLF-assigned genes, i.e. *IL6, Ccl5* and *Crp* are significantly induced in CCl4-exposed mice. In conclusion, a single administration of CCl4 to Abcb4*KO* mice recapitulates several features of ACLF patients, comprising a golden window of survival, advanced liver fibrosis, ductular reaction, massive hepatic necrosis and upregulation of ACLF-related genes. Therefore, the presented mouse model is promising to investigate ACLF pathomechanisms and possible therapeutic interventions.

1.21 Alterations in oncostatin M receptor expression is associated with the progression of non-alcoholic fatty liver disease

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One of the cytokine families most prominently associated with liver inflammation, obesity and metabolic response, is the family of IL-6-type cytokines, in particular IL-6 itself, but also its less well-investigated relative oncostatin M (OSM). IL-6 and OSM have been shown to be intimately connected with our body"s reaction to physical, metabolic and pathogen-induced stress. Reports over the last two decades have clearly pointed out that these cytokines have anti-inflammatory as well as pro-inflammatory signaling activities which very much depend on the cellular and physiological context in which they are released or the receptors they utilize for signaling. Much attention has been given to IL-6 as well as its mode of action, however, recent studies implicate that OSM is much more strongly involved in disease pathogenesis than anticipated so far. Its physiology, however, is far less well understood.

Question OSM can signal via two receptor complexes which might have more pro- or anti-inflammatory activities, respectively. Here, we addressed the question whether alterations in receptor expression become evident in NAFLD progression.

Methods We characterized the expression profile of the type I and type II OSM receptor complex in liver samples from different rodent models of liver inflammation or *in vitro* in hepatoma cell lines stimulated with different inflammatory cytokines using quantitative real-time PCR, Western blot analysis and flow cytometry.

Results We found a distinct decrease of components of the type I OSM receptor complex in livers from mice fed a high-fat diet, after intraperitoneal injection of TNFα or IL-1β or upon cholestasis. At the same time the type II OSM receptor complex appeared to be strongly upregulated. Similar observations were made in HepG2 cells treated with IL-1β. Application of pharmacological inhibitors to prevent activation of distinct mitogen-activated protein kinase pathways (ERK1/2 or p38) demonstrated the necessity of ERK1/2 activity to modulate the expression of the type I OSM receptor complex.

Conclusions Dysregulation of the expression levels of the type I and type II OSM receptor complexes appears to be a conserved feature in the progression of inflammatory liver diseases. Consequently, alterations in OSM receptor levels might be a possible diagnostic marker for the assessment of progressive liver inflammation.

1.22 *Schistosoma mansoni* eggs modulate the host´s hepatic lipid metabolism

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Questions Schistosomiasis (formerly Bilharzia) is a parasitic infectious disease provoked by *Schistosoma mansoni*. The disease is endemic in tropical and recently in temperate climate areas, such as Corsica. The WHO estimates the number of people affected by schistosomiasis > 240 million. Previously, we have shown that schistosomes influence the hepatic lipid metabolism. The present project aims to investigate the metabolic pathways by which *S. mansoni* eggs influence the hepatic lipid metabolism of the host.

Methods Lipid metabolism was analyzed in liver tissue of *S. mansoni*-infected hamsters by western blot (WB), histological staining, mass spectrometry imaging (MSI), and immunohistochemistry (IHC). Non-infected hamsters and hamsters infected with clonal cercariae of one sex without egg production were used as controls. Additionally, human hepatoma cells (HepG2) were stimulated with soluble egg antigens (SEA) isolated from *S. mansoni* eggs.

Results Although the total amount of neutral lipids in the liver of *S. mansoni*-infected hamsters decreases compared to non-infected hamsters, increased lipid accumulation occurs in the eggs and in perigranulomatous hepatocytes. In bisex-infected hamsters, the expression of key enzymes of lipid synthesis such as acetyl-CoA-carboxylase 1 (ACC1) and fatty acid synthase (FAS) was reduced in WB but increased in perigranulomatous hepatocytes. In contrast, enzymes involved in lipid catabolism, e.g. hepatic lipase (LIPC), were downregulated globally but also in hepatocytes close to the granuloma. These findings have been verified *in vitro*.

Conclusion Our data show a change in the composition of neutral lipids between *S. mansoni*-infected and control liver tissue. In addition, we observed increased lipid synthesis and accumulation as well as reduced lipid catabolism in perigranulomatous hepatocytes in the liver of infected hamsters. For the first time, we demonstrate that schistosomal eggs induce a modulation of the host lipid metabolism.

1.23 The dosage matters – divergent effects of IGFBP2 on viability of hepatoma cells

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Background Much progress has been made in hepatocellular carcinoma (HCC) treatment, however, HCC is still the fifth most common cause of cancer-related death worldwide. Therapies still have limited effectiveness and a range of side effects. Understanding of the pathogenesis and underlying molecular mechanisms is of pivotal importance for the development of novel treatment strategies. The family of p53 proteins, (p53, p63, p73 and their isoforms) play an important role in defending cells against toxic stress by transcriptionally regulating specific sets of target genes. We previously identified *IGFBP2* (Insulin like growth factor binding protein 2) as a p73 target gene in HCC. IGFBPs control IGF availability and limit its proliferative effects. Aim of this study was to investigate the impact and physiologic relevance of IGFBP2 induction in HCC cells.

Methods IGF secretion and surface levels of IGF receptors were analyzed in Hep3B cells. The impact of p73 overexpression on IGFBP2 was studied in rAd-TAp73-transfected cells. Effects on p73 and IGFBP2 expression were evaluated after stimulation with HCC therapeutics bleomycin, doxorubicin and regorafenib. Transcriptional regulation was determined by qPCR, protein levels by Western Blot and IGFBP2 secretion by ELISA. Overexpression of *IGFBP2* or treatment with recombinant IGFBP2 was performed to analyze the impact of high doses of IGFBP2.

Results Hep3B cells displayed physiologic IGF secretion and surface expression of insulin receptor and IGF receptor 1. p73 overexpression induced IGFBP2 production and secretion. Treatment of Hep3B cells with HCC-therapeutics resulted in an induction of *TP73* and *IGFBP2* and also led to increased cell death rates. However, only the two chemotherapeutics led to a relevant secretion of IGFBP2 in the range of 500 pg/ml. In contrast, high IGFBP2 concentrations increased cell viability, proliferation and migration and resulted in reduced drug-mediated cell death.

Conclusion Depending on the amount, IGFBP2 has divergent effects in HCC cells: p73 activity induces endogenous IGFBP2 production and secretion in low amounts, which exerts potentially anti-proliferative effects. Contrariwise, high levels of (exogenous) IGFBP2 triggers the proliferative arm of the IGF axis. Thus, fine-tuning of the p73- and IGF-pathways is essential for homeostasis and a detailed understanding might offer new therapeutic options in HCC.

1.24 Correlation of tgfb2 mRNA expression to disease progression in a time course of chronic biliary liver disease

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Question To identify the cellular source of TgfB2 in a time course of cholestatic liver disease using Abcb4-KO mice and PSC patients, and to correlate findings to disease stages.

Methods Liver samples from PSC liver biopsies and Abcb4-KO mice at the age of 2-, 6-, 8- and 12-months were stained for HE, Sirius Red and Orcein to visualize inflammation and fibrosis. Morphological evaluation was performed using Ishak and Nakanuma scoring systems. Tgfb2 mRNA expression was analysed by in situ hybridisation using the RNAscope technique and then compared to age matched wildtype Balb/c mice and disease-free human liver as well as PSC samples.

Results In Abcb4-KO tissues, we found an increase in grade and stage of fibrosis and inflammation with advancing disease progression using both scoring systems in comparison to wild type mice. Disease progression was faster in female than in male mice, especially with regard to inflammation. Subscores, e.g. portal tract inflammation and interface hepatitis increased first, while confluent necrosis occurred not before the age of 12 months. Tgfb2 mRNA was expressed in areas of proliferating bile ducts and fibrotically rearranged tissue at all stages of cholestatic disease. Both murine and human livers showing higher Ishak and Nakanuma scores also showed stronger tgfb2 expression, particularly in samples with a high grade of portal inflammation. We are currently performing costaining with cell type/cell fate markers to specifically identify the cell type that upregulates TGFb2 expression.

Conclusions The expression of tgfb2 mRNA increased with disease progression of Abcb4-KO mice, whereby prominent inflammatory grades present with the highest expression levels in mice and human.

1.25 Low ECM1 expression temporally promote proliferative, tumorigenic and inflammatory gene signatures in mouse liver

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Background We recently provided the first evidence that ECM1 (extracellular matrix protein 1) plays a crucial role in liver homeostasis by attenuating TGF-β activation. ECM1 is often downregulated in liver diseases, including liver cancer and non-alcoholic fatty liver disease. Here we examined the dynamic genomic profile of ECM1 knockout mice liver to define the global molecular changes accompanying its expression depletion.

Methods RNA sequencing was performed in ECM1*KO* mice aged 2, 5 and 8 weeks, representing the initial, intermediate, and advanced phases of the liver disease, respectively. This was accompanied by differentially expressed genes identification and pathway enrichment analysis. Further, to interrogate the link to fibrosis, ECM1 gene expression changes were compared to that derived from activated hepatic stellate cells.

Results ECM1 maintains homeostasis in healthy liver architecture and function. Depletion of ECM1 in mice disturbes liver architecture and causes death at about 8 weeks due to liver failure. There were ~ 2x more differentially expressed genes at the early time point (2 weeks) than at the later (8 weeks) time point, suggesting that most alterations were initiated at the early stage. Livers of ECM1*KO* mice generally showed a notable downregulation of metabolic pathways, and a high expression of cell cycle, proliferation, focal adhesion and PI3K-Akt pathways. While fibrosis signatures persisted from early to the late stage, genes driving pathways in cancer, including hippo, Wnt signaling and ECM-receptor interaction were upregulated at 2 weeks, whereas inflammatory signatures including cytokine-cytokine receptor interaction, chemokine signaling pathway, TNF signaling and Toll-like receptor interaction were the predominant alterations at the 8 weeks time point.

Conclusion Our data suggest that ECM1 suppression temporary provokes tumorigenic signatures at the onset of liver disease and lead to profound inflammatory changes as the disease progresses. These findings pave the way for the characterization of early and late stage signatures driving the initiation and maintenance of liver diseases.

1.26 Hepatocellular Brg1 promotes CCl4-induced liver inflammation, ECM accumulation and fibrosis in mice

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Questions Brahma-related gene 1 (Brg1) is a catalytic subunit of the SWItch/Sucrose Non-Fermentable (SWI/SNF) complex and has recently been identified as important for liver regeneration. Liver fibrosis is a progressive pathological process that involves the depletion of the hepatocellular regenerative capacity and ultimately leads to the development of

liver cirrhosis and even hepatocellular carcinoma. So far the role of Brg1 in liver fibrosis is unclear.

Methods In this study, we examined the effect of Brg1 on the development of liver fibrosis. Hepatocyte specific Brg1 knockout mice (AlbCre Brg1fl/fl) were injected with carbon tetrachloride (CCl4) to induce liver fibrosis. Brg1 expression was determined by Western blot and liver fibrosis was assessed by liver-to-body weight ratio analysis, serum ALT ELISA, Sirius red staining, and alpha-smooth muscle actin (a-SMA) staining.

Results Brg1 expression was significantly increased in the fibrotic liver tissue of wild-type mice compared to untreated wild-type mice. The livers of the Brg1 knockout animals showed a significantly reduced liver inflammation, extracellular matrix accumulation and liver fibrosis compared to wild-type mice. Furthermore, we were able to show that HSC activation and inflammatory response during CCl4-induced liver fibrosis are associated with Brg1, which mediates the TNF-α/NF-kB pathway.

Conclusions These results highlight a new aspect of Brg1 in the pathogenesis of liver fibrosis. We have shown that hepatocyte-specific Brg1 deletion prevents liver fibrosis in CCl4-treated mice. In conclusion, Brg1 promotes the progression of liver fibrosis in mice and therefore can be used as a potential therapeutic target for the treatment of patients with liver fibrosis due to chronic injury.

Correlation of tgfb2 mRNA expression to disease prog

1.27 Sex-specific differences of hepatic metabolism in *Schistosoma mansoni* infection

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Questions Schistosomiasis is one of the most common parasitic infections of humans worldwide with at least 290 million people that required preventive treatment in 2018. During chronic infection, half to two thirds of the parasite´s eggs deposited in mesenteric venules of the mammalian host are swept into the liver. The aim of the study was to investigate gender-specific differences in the dysregulation of hepatic metabolism during *Schistosoma mansoni* infection.

Methods Key enzymes of hepatic metabolism were investigated in liver tissue samples of a hamster *S. mansoni* infection model. Male and female hamsters were either *S. mansoni* bisex-, single sex-, or non-infected. Liver samples were analyzed by western blotting and malondialdehyde assay (MDA).

Results While rate limiting enzymes of glycolysis were upregulated, gluconeogenesis, glycogenesis, pentose phosphate pathway, and fatty acid synthesis were reduced in *S. mansoni*-bisex-infected hamster livers of both gender without differences. Further, we observed increased oxidative stress in the livers of bisex-infected female hamsters compared to single-sex or non-infected hamsters whereas in male hamsters no group differences occurred. Markers of late hepatic autophagy were generally upregulated in bisex-infected female hamsters and downregulated in bisex-infected males compared to single-sex- or non-infected control groups.

Conclusion We demonstrated that *S. mansoni*-infection leads to a dysregulation of the carbohydrate and lipid metabolism in the liver of hamsters without gender-specific regulatory differences. However, *S. mansoni* induced oxidative stress in the liver of female hamsters but not in males. In line with these findings, we detected an upregulation of autophagy in females. It remains unclear, however, whether autophagy can excert a protective effect or instead contribute to the pathogenesis of schistosomiasis. For the development of novel therapeutic approaches and diagnosis of liver disease, it should be taken into consideration that autophagy could represent a key and critical factor in hepatic injury.

1.28 Aging impairs hepatic stellate cell functions

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Hepatic stellate cells are liver-resident mesenchymal stem cells residing in a quiescent state on a basement membrane-like structure in the space of Disse, which represents their perivascular niche. One component of this niche is the laminin-α5 (LAMA5) chain, which constitutes heterotrimeric laminin-521 (LN-521) together with the LAMB2 and LAMC1 chains. LN-521 sustains quiescence and hepatocyte growth factor (HGF) expression in stellate cells. During aging, hepatic stellate cells acquire a senescence-associated secretory phenotype characterized by increased expression of inflammatory factors and reduced expression of growth factors such as HGF. Mechanical forces are reduced in sinusoids of the aged liver by declined hepatic blood flow of about 50 % as reported earlier. Exposure of isolated stellate cells to fluid shear stress triggers HGF release in an integrin-α5/β1 (ITGA5/ITGB1) dependent manner and increases *Itga5*, *Lamb2*, and *Lamc1* expression. These laminin chains, ITGA5, ITGB1, and HGF decrease in the aged rat liver. Interestingly, CRISPR/Cas9-mediated knockout of ITGA5 and ITGB1 significantly lowered HGF release by stellate cells. Thus, ITGA5/ITGB1 heterodimer is important for the sensing of mechanical stimuli by stellate cells, a process that is disturbed by aging. In conclusion, the reduction of extracellular matrix proteins such as LN-521 in the whole liver and lowered expression of ITGA5 and HGF in stellate cells is most likely induced by the regression of mechanical forces in the aged liver. Impaired integrin-mediated anchorage and quiescence of stellate cells indicate disturbed integrity of their niche in the space of Disse during aging, which results in alleviation of stellate cell functions such as HGF synthesis and release. Age-related alterations in stellate cells offer explanations for the declined regenerative potential of the aged liver since stellate cells are important sources of trophic factors involved in liver maintenance and repair.

1.29 Recurrent intrahepatic cholestasis of pregnancy in conjuntion with a frameshift deletion in FGFR4

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Intrahepatic cholestasis of pregnancy (ICP) is the most common liver disease in pregnant women. ICP usually becomes apparent in the third trimester of pregnancy. The condition resolves usually after delivery, and clinical signs and symptoms disappear. ICP is caused by a multifactorial etiology with hormonal, environmental and genetic factors contributing to the development of the disease. To date, causal variants in hepatobiliary ATP-binding cassette transporters for phosphatidylcholine (ABCB4) and bile acids (ABCB11) have been identified. Here, we present the case of a 29-year old women with recurrent ICP and severe pruritus in two consecutive pregnancies. Exome sequencing (NGS) revealed a previously not described deletion (c.393_delG) in the Fibroblast growth factor receptor 4 (*FGFR4)* gene causing a frameshift and a dysfunctional protein. Pathogenic *ABCB4* and *ABCB11* mutations were not detected. FGFR4 is the hepatic receptor for the enterokine FGF19 that represses bile acid synthesis. Hence, we speculate that the *FGFR4* variant interferes with the enterohepatic FGF19 feedback loop and thus predisposes to ICP. This is the first ICP case due to an *FGFR4* mutation described to date.

Poster Visit Session II Clinical Hepatology, Surgery, LTX
Friday, January 29, 2021 2:40 pm – 3:25 pm, Poster Session Virtual Venue

2.4 2D Shear Wave Elastography Predicts Survival in Advanced Chronic Liver Disease: AIXPLORER PREDICT and VALIDATE

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Background and Aims Measurement of liver stiffness (LSM) by 2-dimensional shear wave elastography (2D-SWE) is an established technique to assess significant fibrosis and clinically significant portal hypertension. The aim of this multicenter retrospective study was to assess the value of LSM by 2D-SWE (L-SWE) to predict outcome in advanced chronic liver disease (ACLD) patients.

Method This international multi-center cohort study included patients with ACLD, valid L-SWE at baseline and at least 28-day follow-up. More than 40 % of patients were also recorded of LSM by transient elastography (TE). Clinical and laboratory parameters at baseline as well as complications during follow-up were recorded. An external validation cohort was also established of patients with pSWE measurements. The primary outcome was overall mortality of the patients. The secondary outcome was worsening or development of any decompensation episode.

Results After screening 2,148 patients from 16 centers, 1,827 patients were included. The median age of the study population was 55 years, with approx. 62.4 % males. The median L–SWE was 11.8 kPa (interquartile range: 7.4–24.5). Apart from the MELD score, L-SWE is an independent risk factor of mortality with an AUC of 0.80 (95 %CI: 0.76-0.83). A cut-off of 20 kPa in L-SWE combined with a cut-off of 10 in the MELD score could stratify risk of mortality and development of decompensations. The two-year mortality and decompensations rates were 36.9 % and 61.8 %, respectively, in high-risk patients with L-SWE ≥ 20 kPa and MELD≥10, whereas mortality and decompensation rates were 1.1 % and 3.5 %, respectively, in low-risk patients with L-SWE < 20 kPa and MELD < 10 (M10S20). The M10S20 algorithm was also confirmed with the TE measurement and in external validation cohort for predicting risk of decompensations development and the risk of mortality.

Conclusion This study delivers an easy algorithm for the stratification of patients with ACLD based on L-SWE and MELD score. Patients with L-SWE≥20 kPa combined with MELD score≥10 should be followed closely and receive more intensified care, while patients with low risk can be managed at longer intervals.

2.5 2D shear wave elastography and risk for de novo hepatocellular carcinoma in patients with advanced chronic liver disease

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Background and Aims Liver cirrhosis is the most important risk factor in the development of de novo hepatocellular carcinoma (HCC) regardless of etiologies. Early non-invasive screening for cirrhosis using liver stiffness (LS) may also give information on the risk of HCC. This is the large-scale international multicenter study to evaluate clinically relevant end-point in liver cirrhosis using two-dimensional real-time shear wave elastography (L-SWE).

Method Chronic liver disease patients were screened from 16 centers from Europe and China. Besides, they were included if they had valid 2D-SWE at baseline. Clinical and laboratory parameters were assessed at baseline. We conducted the follow-up regularly after first assessment. De novo HCC was diagnosed according to the 2018 Practice Guidance by the American Association for the Study of Liver Diseases. Univariate and multivariate logistic regression model were used to explore risk factors of de novo HCC. Area under the receiver operating characteristic curve (AUROC) and Youden index were analyzed to find out the optimal cutoff for LS with SWE.

Results A total of 1881 were included in the study with a median follow-up of 2.3 years. Eighty-five (4.5 %) patients were diagnosed with de novo HCC, and had a doubled median 2D-SWE value than those without HCC (22.0 vs. 11.1, p < 0.001). L- SWE (OR: 1.013, p=0.022) was independently associated with developing de novo HCC in multivariate analysis. Age, gender and platelets count were also found independent risk factors for de novo HCC development. The AUROC of L-SWE combined model was 0.756 (95 %CI: 0.716 - 0.796), which was significantly higher than the MELD and Child-Pugh scores in predicting de novo HCC. And the model fits good in the calibration plot. At the best cut-off of 15kPa, a 77 % of sensitivity and a 63 % of specificity were obtained, with a negative predictive value of up to 98 %. Patients with L-SWE values above the cutoff (≥ 50 kPa, OR:3.866, p < 0.001) and lower platelet counts (< 170G/L) were markedly associated with de novo HCC. A cut-off value of 50 years old and male sex were also established for the risk stratification algorithm.

Conclusion The L-SWE is associated with de novo HCC with the optimal cutoff of 15 kPa, combined with PLT of 170G/L, age of 50 and male sex. Three significantly different de nove HCC risk groups could be classified. The algorithm could be used as a convenient diagnostic tool to stratify the risk of de novo HCC.

2.6 Long-Term Efficacy and Safety of Obeticholic Acid in Patients With PBC From POISE Grouped by Risk of Disease Progression

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Question Primary biliary cholangitis (PBC) is a rare liver disease characterized by chronic cholestasis, fibrosis, and cirrhosis. Obeticholic acid (OCA) is approved as second-line treatment for PBC in patients who are incomplete responders or intolerant to ursodeoxycholic acid. Elevated alkaline phosphatase (ALP) and bilirubin levels predict long-term outcomes in PBC. We evaluated the long-term efficacy and safety of OCA in subgroups from the POISE trial categorized biochemically by risk of disease progression.

Methods The phase 3, randomized, double-blind, 12-month POISE trial evaluated OCA vs placebo in patients with PBC; a 5-year open-label extension followed. We evaluated OCA efficacy and safety in 2 patient subgroups based on baseline biochemical status: ALP ≤3X upper limit of normal (ULN) vs ALP > 3X ULN and total bilirubin ≤ULN vs total bilirubin > ULN. OCA response was ALP < 1.67X ULN, total bilirubin ≤ULN, and ALP decrease of ≥15 % from OCA baseline.

Results Both subgroup analyses included 193 patients (ALP ≤3X ULN = 142; ALP > 3X ULN = 51; total bilirubin ≤ULN = 172; total bilirubin > ULN = 21). Mean (SD) ALP levels (U/L) at OCA baseline were 257.0 (45.8) in the ALP ≤3X ULN

group and 484.5 (103.4) in the ALP >3X ULN group. Mean total bilirubin levels (µmol/L) at OCA baseline were 9.6 (4.0) in the total bilirubin ≤ULN group and 27.3 (6.0) in the total bilirubin >ULN group. Mean change from baseline was –266.3 U/L in the ALP >3X ULN group versus –63.9 U/L in the ALP ≤3X ULN group at month 72. Total bilirubin levels remained stable within the total bilirubin ≤ULN group and decreased in the total bilirubin >ULN group (▶**Figure**). Pruritus was the most frequently reported adverse event in all 4 subgroups (88 % in the ALP >3X ULN group; 74 %–78 % in other groups). Discontinuations due to pruritus were 3 %–8 % in both ALP and the total bilirubin ≤ULN groups; none occurred in the total bilirubin >ULN group.

Conclusions OCA treatment was safe and efficacious and resulted in durable improvements in markers of hepatic injury and cholestasis, regardless of baseline ALP and total bilirubin levels. Strong biochemical improvements with OCA were achieved in patients with elevated levels of ALP and bilirubin.

Figure. Mean (SD) Change From OCA Baseline to Month 72 in ALP and Total Bilirubin in Patients Grouped Biochemically by Risk of Disease Progression

2.7 Percutaneous transhepatic cholangiodrainage in patients with PSC: a multicentre, retrospective analysis

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Background and Aims Endoscopic retrograde cholangiography (ERC) is the method of choice to treat biliary strictures in patients with primary sclerosing cholangitis (PSC). However, in a subgroup of PSC patients, biliary stenoses are complex and endoscopic biliary drainage cannot be established using ERCP. There is no generally accepted recommendation how to proceed in this situation, highlighting a severe lack of data. The aim of this study was to investigate the risks, benefits and clinical outcomes of PSC patients after percutaneous transhepatic cholangiodrainage (PTCD) in an international, multicentre study.

Method Retrospective patient data was provided from four European centres. Medical records were reviewed for clinical data including laboratory results, previous endoscopic interventions, and periinterventional complications.

Results A total of 37 PSC patients who underwent PTCD were identified. Strictures were localised in the common bile duct (CBD) in most of the cases (CBD=51.4 %; bifurcation = 16.2 %; left=13,5 %; right=2,7 %; multiple=16.2 %). Liver cirrhosis was present in 14 patients (37.8 %) with a median MELD score of 16 at the time of first PTCD. In most cases jaundice (51.2 %) was the indication for PTCD, followed by recurrent bacterial cholangitis (16.3 %), suspected cholangiocarcinoma (CCA, 16.3 %) and pruritus (11.6 %). Mild periinterventional complications occurred in 20 patients (54 %; 19x bacterial cholangitis, 1x pancreatitis). Severe complications in three patients (bile duct perforation, dislocation of catheter, pleural puncture) resolved without sequelae. Overall, total bilirubin and CRP levels significantly decreased six months after PTCD (▶**Fig. 1**). Pruritus improved in four of five patients after PTCD.

Conclusion To our knowledge, this is the first multicentre study investigating clinical outcomes of PSC patients undergoing PTCD. The procedure was generally safe and mild complications prevailed. PTCD for PSC patients is effective by amending jaundice and pruritus and by improving biochemical signs of cholestasis and inflammation. These results indicate that PSC patients who lack endoscopic options for biliary drainage benefit from PTCD.

Figure 1 Total bilirubin and CRP before (median bilirubin 5.44 mg/dl, CRP 30.5 mg/dl) and six months after PTCD (median bilirubin 1.08 mg/dl, CRP 4 mg/dl). * = $p < 0.05$.

2.8 LPS dosage-dependently regulates FOXA2 expression through NFkB signaling and thus determining BSEP expression in ACLF

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Background & Aims Apical ABC-transporter BSEP/ABCB11 is in charge of bile acid delivery. Loss of BSEP in end-stage liver disease such as acute-on-chronic liver failure (ACLF) leads to severe cholestasis and poor prognosis. Our previous study shows that transcription factor FOXA2 replaces FXR to control BSEP expression in ACLF. As a sepsis-like syndrome, ACLF patients usually suffer from severe bacterial infection. In this study, we scrutinize how LPS influences apical BSEP expression through modulating FOXA2.

Methods We investigated 18 ACLF patients, 13 receiving liver transplantation (LTx) and 5 recovered. BSEP and FOXA2 were examined in liver tissues by immunohistochemistry. LPS effects on FOXA2 and BSEP expression were investigated in cultured hepatocytes and in mice.

Results Recovered ACLF patients display apical BSEP expression in hepatocytes. In those receiving LTx, 3 patients lacking BSEP expression show more severe disease. Compared with irreversible patients possessing BSEP, the patients who lost apical BSEP demonstrated higher serum total bilirubin concentration, higher international normalized ratio and Model for End-stage Liver Disease score. 15 patients who maintained apical BSEP expressed robust FOXA2 in hepatocytes. ChIP assays confirmed that FOXA2 initiated *ABCB11* transcription through binding to the gene promoter in primary human and mouse hepatocytes. Foxa2 and Bsep expression was induced with 2-5 mg/mL LPS in hepatocytes. Knockdown of Foxa2 by RNAi abolished LPS-induced Bsep expression. ChIP assays showed that LPS increased binding activity of Foxa2 to the *bsep* gene promoter. ChIP analyses further revealed that LPS treatment led to binding of p65, a NFkB signaling protein, to the *foxa2* gene promoter. When hepatocytes were treated by p65 nuclear translocation inhibitor JSH23, p65 binding to the *foxa2* gene promoter was blocked and expression of Foxa2 and Bsep were inhibited. Interestingly, high concentrations of LPS (20mg/mL) inhibited Foxa2 nuclear translocation and induced high levels of p-Foxa2, which resulted in inhibition of BSEP expression. Consistent with the *in vitro* observation, regulation of nuclear FOXA2 and BSEP expression in hepatocytes by LPS is dosage dependent in mice

Conclusions LPS dosage-dependently regulates FOXA2 expression through NFkB signaling and thus determining BSEP expression in severe inflammatory diseases, such as ACLF.

2.9 First year experience of liver graft normothermic machine perfusion at University hospital Münster

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Introduction Normothermic machine perfusion (NMP) of allografts is the most exciting next evolutionary step in solid organ transplantation. Using NMP harmful cold storage times can be reduced, hazardous reperfusion injury will be diverted from the recipient onto the machine and logistics of solid organ transplantation will shift from an emergency setting into a plannable procedure. Additionally, functionality testing prior transplantation will render liver transplant to a safer intervention avoiding early graft dysfunction as well as primary non-function (PNF).

Methods 22 postmortal donor liver allografts with a median donor age of 59.3 years, median donor BMI of 27.4 kg/m^2 and median DRI of 1.7 were connected onto NMP in Münster University Hospital since October 2019. Average cold ischeamia times was 6.5 hours before graft perfusion on the OrganOx metra device. Median NMP time was 12 hours. Perfusion with physiological pressure and flow rates wasconducted via portal vein and hepatic artery with three packages of packed red blood cells and 500 ml Gelatine-Polysuccinat (Gelafundin® 4 %). Selecting criteria for NMP was estimated time of arrival of the graft, quality concerns of grafts being suitable for perfusion or recipient issues such re-transplantation.

Results Since implementation of NMP in Münster University Hospital in October 2019 22 of 47 (47 %) accepted donor livers were perfused normothermically. Except of one liver allograft all NMP livers showed sufficient lactate clearance (lactate levels < 3 mmol/l at 1-3 hours of NMP perfusion). In most patients transaminases peaked during NMP and there was no severe reperfusion syndome in recipients. Primary graft function was 95.5 %, one organ revealing insufficient lactate clearance on NMP went into PNF.

Conclusions Viability testing of poor quality, extended criteria donor livers using NMP has the potential for increasing the number of transplantable donor organs and thus faces the problem of organ shortage without jeopardizing recipient safety. Additionally, NMP can turn liver transplantation into a plannable, non-emergency procedure, allowing better teaching and training conditions.

2.10 Assessment of liver allograft viability during *ex vivo* normothermic machine perfusion using the MS^2 score

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Introduction Utilization of extended criteria donor organs becomes more and more frequent in order to counteract waitlist mortality. Normothermic machine perfusion (NMP) facilitates checking liver allografts for their viability and has the scope to improve graft quality. A variety of perfusion criteria have been reported previously to assure graft viability. After establishing NMP at University Hospital Muenster we aim to critically evaluate and simplify existing assessment scores and would like to introduce the MS^{2}score (Münster machine score).

Methods In total 22 NMP of liver grafts were performed from 10/2019 until 10/2020. During graft perfusion portal- and arterial flow, perfusate pH, lactate and glucose levels as well as production of bile, bile viscosity and pH were monitored hourly. A minimum of 4 hours of NMP was local protocol to allow the graft to recover from static cold storage. Perfusate transaminases were monitored at 1, 4, 8, 12 and 16 hours of NMP. Following transplantation, serum lactate levels, clotting factors and transaminases of the recipient were monitored daily.

Results Average DRI was 1.7 despite rather short average cold ischemia time of 6,5 hours. Average NMP lasted 12 hours. Median recipient MELD was 26. 21 transplants were successful with 95,5 % primary function. There was no EAD beyond postoperative day 3, except for 3 cases (13 %) with elevated bilirubin levels for >7 days. Using previously reported viability variables such as transaminases >6000 U/l and perfusate pH<7.2 we would have excluded 9 livers. Applying the Cambridge glucose metabolism criteria 7 additional grafts would have been excluded. Relying on perfusate lactate level (<3 mmol/l @ 4 h), arterial flow (>150 ml/min or >20 % of total perfusion volume) and production of viscous bile was sufficient for viability assessment. The presented simplified MS2score seems to be sufficient for viability testing livers on NMP including a significant proportion of grafts which elsewhere would have been excluded.

Conclusion The MS^2 score provides a simple and therefore ideal tool for viability assessment of livers on NMP. Previously reported viability criteria might have been chosen too carefully and would have excluded significant portion of functioning liver allografts.

2.11 Detection of PD-L1$^{(+)}$Circulating Tumor Cells Significantly Correlates with Postoperative Recurrence in HCC Patients

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Background/Purpose Hepatocellular carcinoma (HCC) is the fifth most common tumor worldwide. Circulating tumor cells (CTCs) are associated with an increased risk of recurrence. Programmed death ligand 1 (PD-L1) is associated with increased tumor aggressiveness. The aim of this work is to evaluate the potential of CTCs with PD-L1 expression (PD-L1$^{(+)}$CTCs) as prognostic markers in HCC.

Methods Blood samples from 28 patients with HCC who underwent tumor resection were analyzed. Venous blood was taken before and immediately after the surgery to isolate and quantify PD-L1$^{(+)}$CTCs by flow cytometry. Hepatitis and HIV infection were an exclusion criterion in this study.

Results Twelve HCC patients (42.8 %) had liver cirrhosis. The median overall survival was 27.3 months. The median recurrence-free survival was 15.8 months. PD-L1$^{(+)}$CTCs (21.4 %, 0.5 PD-L1$^{(+)}$CTC/ml) could be identified in 6 patients after the surgery. The postoperative detection of PD-L1$^{(+)}$CTCs correlated with tumor aggressiveness and shorter recurrence-free survival ($p = 0.002$) as well as with shorter overall survival ($p = 0.050$).

Conclusion The postoperative detection of PD-L1$^{(+)}$CTCs correlates with tumor aggressiveness and postoperative recurrence in human hepatocellular carcinoma. The adjuvant administration of checkpoint inhibitors in HCC patients with positive PD-L1$^{(+)}$CTCs could represent an interesting approach for clinical studies.

2.12 Testing for Antibody-Induced BSEP Deficiency (AIBD) using a Novel Cell-based Assay

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Background Antibody-induced bile salt export pump (BSEP) deficiency (AIBD) is an aquired type of intrahepatic cholestasis that may arise after orthotopic liver transplantation for PFIC-2 in patients with compete absence of BSEP expression. IgG-type antibodies are formed against the transplanted alloantigen which bind to BSEP from the canalicular lumen, effectively causing recurrence of pre-transplant symptoms by BSEP trans-inhibition. We devised and validated a simple robust assay for measuring BSEP trans-inhibition by patients" serum antibodies to functionally confirm AIBD diagnosis.

Methods Human embryonal kidney (HEK) 293 cell lines stably expressing either human NTCP-mCherry, BSEP-EYFP, or both were generated by lentiviral transduction and clonal isolation. Patient sera were tested for BSEP antibodies by western blot and immunofluorescence staining of human liver and HEK293-BSEP-EYFP cells. Using our cell lines, a two-phase assay was devised consisting of preloading cells by NTCP with [^{3}H]-Taurocholate (TC) followed by TC export into fresh medium by BSEP. To test for BSEP

trans-inihibition, NTCP/BSEP-expressing cells were pre-incubated with either AIBD or control sera prior to the assay. Live cell staining was performed to demonstrate extracellular serum IgG binding to BSEP and exclude BSEP internalization upon IgG binding.

Results and Conclusion Comparison of the stable cell lines demonstrated specific [^{3}H]-TC uptake during the import phase dominated by NTCP and excretion during the export phase by BSEP in which re-import of [^{3}H]-TC by the sodium-dependent NTCP was prevented by substituting choline for extracellular sodium. Thus, BSEP inhibition by sera from an AIBD patient cohort as well as by purified AIBD antibodies, but not by control or non-AIBD cholestatisis cohorts could be demonstrated. Our data show that the assay readout results from direct functional BSEP inhibition and is not impaired by serum complement activation or BSEP internalization upon antibody binding. In contrast, excessive levels of serum bile salts need to be removed before the assay as they compete with the model substrate. Notably, we found high anti-BSEP titers in one asymptomatic post-OLT PFIC-2 patient, which showed no trans-inhibition *in vitro*. This clearly shows that detection of anti-BSEP antibodies alone is insufficient for AIBD diagnosis but needs to be complemented by a functional inhibition test.

2.13 Der Stellenwert des portal-hepatischen Druckgradienten in der Therapie des refraktären Aszites durch TIPS

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Hintergrund Bei der Leberzirrhose ist die Erhöhung des hepatischen Widerstands und damit des portal-hepatischen Druckgradienten (PHPG) ursächlich für die Entwicklung von Varizen und Aszites. Hierbei ist die Anlage eines transjugulären intrahepatisch portosystemischen Shunts (TIPS) zur Senkung des PHPG indiziert als Therapieoption der Varizenblutung und des refraktären Aszites. Während bei der Varizenblutung der Ziel-PHPG gut untersucht ist, wird dieser bei der Behandlung refraktären Aszites noch kontrovers diskutiert.

Methodik In dieser Studie wurden die prä- und post- TIPS gemessenen Druckgradienten von 341 Patienten, welche eine TIPS-Anlage zwischen November 1992 und Juli 2015 zur Behandlung therapierefraktären Aszites erhielten, mit dem Überleben und der Aszitekontrolle korreliert.

Resultate Eine schwere portale Hypertension vor TIPS Anlage (PHPG größer 19mmHg) war ein Prädiktor für ein reduziertes Überleben der Patienten (HR 1.4 [1.0-1.8]; P=0.03). Der PHPG war bei Patienten, welche nach 6 Wochen keinen Aszites mehr aufwiesen prozentual stärker gesenkt worden, als bei Patienten mit weiter punktionswürdigem Aszites (Mittelwert -63 % vs. -54 % des initialen PHPG; P=0.003). Multivariable Analysen zeigten unabhängige Assoziationen zwischen dem Vorliegen von Aszites 6 Wochen post-TIPS und einer PHPG Reduktion < 60 % bei TIPS-Anlage (OR 0.6 [0.4-1.0]; P=0.03), dem initialen Child-Pugh Score (OR 1.3 [1.0-1.6]; P=0.03), dem initialen Vorliegen eines ACLF (OR 2.0 [1.2-3.4]; P=0.005) und invers den initialen Serum-Natriumwerten (OR 0.9 [0.9-1.0]; P=0.002). Zudem zeigte sich ein signifikant längeres Überleben bei Patienten ohne Aszites in der Kontrolle 6 Wochen nach TIPS-Anlage (Medianes Überleben: Aszites 18 Monate vs. kein Aszites 41 Monate; HR 1.5 [1.1-1.9]; P=0.02).

Schlussfolgerung Bei der TIPS-Anlage zur Behandlung therapie-refraktären Aszites könnte eine PHPG-Senkung ≥60 % eine Verbesserung der Aszites Kontrolle und damit des Überlebens erzielen. Eine strukturierte sonographische Nachsorge 6 Wochen nach TIPS-Anlage, scheint zudem sinnvoll zur Detektion von Patienten mit erhöhtem Mortalitätsrisiko.

2.14 *HSD17B13* and *MBOAT7* as modulators of *PNPLA3*-associated cirrhosis

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Background Carriers of the adiponutrin (PNPLA3) p.I148M variant, and in particular homozygous patients, have an increased risk for progression of chronic liver diseases and cirrhosis. In addition, a hepatoprotective splice variant of *HSD17B13* reduces the cirrhosis risk (Abul-Husn et al. N Engl J Med 2018), whereas mutations in the *MBOAT7* gene aggravate liver injury. Our aim was to investigate how the *HSD17B13* and *MBOAT7* variants modulate the risk for an advanced stage of cirrhosis in homozygous carriers of the *PNPLA3* variant.

Methods In total, 998 patients with cirrhosis were screened for the *PNPLA3* variant. Clinical, laboratory and imaging data were collected. Decompensation was defined as the presence of ascites, prior variceal bleeding, hepatic encephalopathy, or jaundice (serum bilirubin ≥ 3.0 mg/dl). Within this cohort, *MBOAT7 (*rs641738) and *HSD17B13* (rs72613567) variants were genotyped by PCR-based allelic discrimination assays, and genotype-to-phenotype correlations were analyzed.

Results Overall, 148 patients (15 %) were homozygous carriers of the *PNPLA3* p.148M/M genotype (64 % men, age 30–81 years). Causes of cirrhosis resembled a typical European population (59 % alcoholic, viral 14 %, NASH 9 %, 14 % other etiologies). Two thirds of the patients were in the decompensated stage of the disease. When comparing patients according to the presence or absence of the protective *HSD17B13* variant, 36 % were carriers in the decompensated stage versus 56 % in the compensated stage (p=0.025). Similarly, MELD scores (13.1 versus 10.9; p=0.04), Child-Pugh score (7.6 versus 7.1; p=0.23) and bilirubin concentrations (3.3 versus 1.7 mg/dl, p=0.035) where significantly higher when the *HSD17B13* variant was absent, contributing to the advanced stage of cirrhosis. These parameters were not influenced by *MBOAT7* alleles.

Conclusions The risk of progressive cirrhosis is increased in homozygous carriers of the *PNPLA3* genotype p.148M/M. Notably, the presence of a *HSD17B13* allele mitigates, at least in part, the harmful effects conferred by *PNPLA3* homozygosity. As both PNPLA3 and HSD17B13 localize to hepatic lipid droplets, the functional interaction might occur at this intracellular localization.

2.15 Obeticholic Acid Efficacy in Patients With NASH Monitored Using Noninvasive Tests: Post Hoc Analysis of REGENERATE Trial

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Question Liver biopsies are not practical for monitoring patients with NASH. Non-invasive tests (NITs) are effective tools for diagnosis and evaluation of these patients. Obeticholic acid (OCA) is an antifibrotic in clinical development for the treatment of advanced fibrosis due to NASH. This post hoc analysis evaluated NIT-based OCA efficacy in patients from REGENERATE stratified by fibrosis stage using NITs.

Methods The phase 3, multicenter, randomized, double-blind REGENERATE trial is ongoing worldwide. This post hoc analysis evaluated the NIT-based efficacy of OCA 25 mg vs placebo (PBO) in patients from the intent-to-treat (ITT) population of the REGENERATE 18-month interim analysis with Fibrosis-4 (FIB-4) and transient elastography (TE) data available at baseline. Using published cutoffs, FIB-4 and TE were applied sequentially to categorize patients. Indeterminate patients scored indeterminate on FIB-4 (≥1.30–<2.67) and TE (≥7.9–<9.6 kPa); advanced fibrosis patients scored advanced on FIB-4 (≥2.67) *or* indeterminate on FIB-4 (≥1.30–<2.67) and as advanced fibrosis on TE (≥9.6 kPa) at baseline. Alanine aminotransferase (ALT) levels, FIB-4, and TE scores were evaluated at baseline and at months 6, 12, and 18 in patients treated with OCA 25 mg or PBO; patients with indeterminant status for advanced fibrosis or advanced fibrosis were pooled.

Results Identified were 543 evaluable NASH patients (OCA 25 mg, n = 266; PBO, n = 277). In the OCA 25 mg and PBO groups, respectively, similar percentages of patients had indeterminate status for advanced fibrosis (6 % and 5 %) or advanced fibrosis (39 % and 37 %). In the pooled subgroups (indeterminate status + advanced fibrosis), OCA 25 mg treatment reduced ALT below the upper limit of normal for REGENERATE (55 U/L) by month 6 and through month 18 (**Figure**). Changes from baseline to month 18 in mean ALT and median TE scores in the pooled subgroups of patients were numerically greater for OCA 25 mg vs PBO, respectively (ALT: –46.1 U/L vs –29.3 U/L; TE: –3.4 vs –0.55 kPa). In the pooled subgroups at month 18, OCA 25 mg reduced mean ALT scores and median TE scores by 50.1 % and 25.6 %; reductions for PBO were 30.2 % and 4.2 %, respectively.

Conclusions Patients with indeterminate status for advanced fibrosis or advanced fibrosis stratified by baseline NITs in REGENERATE showed improvements in NIT measures with OCA 25 mg vs PBO at month 18. These data support the use of routinely available NITs for monitoring OCA treatment response.

2.16 8-oxo-7,8-dihydro-guanosine-urine levels are elevated in patients with liver cirrhosis and hepatic encephalopathy

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Background Hepatic encephalopathy (HE) is a frequent complication in liver cirrhosis and oxidative stress plays an important role in its pathogenesis. A self-amplifying cycle of astrocyte swelling and oxidative as well as nitrosative stress leads to the alteration of neuronal function and the clinical symptoms of HE. 8-oxo-7,8-dihydro-guanosine (8-OHG) is a marker for oxidative stress, that can be measured in urine.

Aim Evaluation of 8-OHG as a marker for oxidative stress in patients with liver cirrhosis and HE

Study design Until now 64 Patients were included: 34 with liver cirrhosis and HE, 2 with liver cirrhosis and no HE, 10 controls with no known liver disease, 12 patients with various tumor diseases and 6 patients with pneumonia and no liver disease.

Hepatic encephalopathy was assessed using a computer based testing battery (Wiener Testsystem, Schuhfried GmbH, Wien), that is well established at our clinic. Furthermore the critical flicker frequency (CFF) of all patients was measured using the Hepatonorm analyzer (nevoLAB®, Meyerhöfen). From all patients with liver cirrhosis blood and urine samples were taken and analyzed. Blood-ammonia levels were measured in the routine diagnostics in the central laboratory of the university hospital Düsseldorf. 8-OHG-levels were measured in urine by mass spectroscopy.

Results Patients with liver cirrhosis and HE have statistically significant higher levels of 8-OHG in urine then patients with liver cirrhosis and no HE or controls without liver disease (3,55 µmol/mol creatinine vs. 2,16 µmol/mol creatinine vs. 2,20 µmol/mol creatinine). The levels of 8-OHG in patients with liver cirrhosis and HE were comparable with the levels in tumor patients or patients with pneumonia. Furthermore we identified a trend towards a negative correlation between 8-OHG levels and CFF. In addition our data show a significant negative correlation between 8-OHG and the serum levels of the antioxidant uric acid.

Discussion Our data indicate that 8-OHG is a marker for hepatic encephalopathy in patients with liver cirrhosis. Moreover higher levels of 8-OHG seems to be connected to more severe episodes of HE. Further experiments will investigate a potential decrease of 8-OHG levels in patients recovering from an episode of HE.

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2.17 Leberphänotyp bei Erwachsenen mit compound-heterozygotem Alpha1-Antitrypsin-Mangel (Genotyp Pi*SZ)

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Hintergrund Bei der klassischen Form des Alpha1-Antitrypsin-Mangels (Genotyp Pi*ZZ) ist die Prädisposition zur Entwicklung einer Lungen-und Lebererkrankung gut charakterisiert. Das Ausmaß der Leberbeteiligung bei dem noch häufiger vorkommenden compound-heterozygoten Genotyp Pi*SZ ist dagegen unklar. Daher untersuchten wir den Phänotyp von Pi*SZ- und Pi*ZZ-Individuen in einer internationalen Kohorte.

▶**Tab. 1** Charakteristika von Pi*SZ-Probanden im Vergleich zu Pi*ZZ- und Pi*MM-Individuen.

	Pi*MM n = 279	Pi*SZ n = 190	Pi*ZZ n = 586	p-Wert Pi*SZ vs. Pi*MM	p-Wert Pi*SZ vs. Pi*ZZ
Alter (Jahre)	52.4±14.6	50.8±15.7	54.2±13.2	.249	**.008**
Geschlecht (% weiblich)	49	53	46	.453	.126
BMI (kg/m²)	25.6±4.5	26.3±5.2	25.0±4.4	.113	**.002**
AAT Serumlevel (mg/dl)#	139.5±25.1	63.4±20.6	28.3±16.0	**<.0001**	**<.0001**
Lebersteifigkeit (kPa)	4.6±1.6	5.2±2.5	6.6±5.2	**.001**	**<.0001**
ALT (% ULN)	65.9±29.8	74.1±51.7	78.8±47.6	.051	.258
AST (% ULN)	62.5±22.5	70.4±40.0	74.1±31.1	**.018**	.216
GGT (% ULN)	57.7±45.7	93.6 ±140.1	96.7 ±122.4	**.003**	.792
AP (% ULN)	58.5±18.5	70.7±36.3	66.1±23.6	**<.0001**	.135

Methodik Im Rahmen einer multizentrischen Studie wurden 190 Pi*SZ-Individuen, 586 Pi*ZZ-Subjekte und 279 Kontrollen (Genotyp Pi*MM) aus neun europäischen Ländern (Deutschland, Österreich, Belgien, Dänemark, Großbritannien, Italien, Spanien, Portugal, Polen) und den USA prospektiv rekrutiert. Alle Teilnehmer erhielten eine standardisierte klinische sowie laborchemische Untersuchung, inklusive einer nichtinvasiven Lebersteifigkeits-Messung (LSM). Leber-Komorbiditäten und pathologischer Alkoholkonsum wurden ausgeschlossen.
Ergebnisse Quantitative Werte wurde erfasst als Mittelwert ± Standardabweichung und relative Häufigkeiten (%). Abkürzungen: ULN, upper limit of normal (geschlechts-spezifisch). # Serumspiegel bei nicht-augmentierten Individuen.
Verglichen mit der Populationsfrequenz (Pi*SZ 1:500, Pi*ZZ 1:2000) waren Pi*SZ-Individuen in unserer Kohorte deutlich unterrepräsentiert. Es zeigte sich bei Pi*SZ-Trägern ein mittlerer Serumspiegel von AAT. Außerdem präsentierten sich Pi*SZ-Individuen mit signifikant erhöhter Lebersteifigkeit im Vergleich zu Kontrollen, jedoch mit niedrigeren Werten als Pi*ZZ-Probanden. Bei den Leberwerten zeigten sich signifikant höhere AST-, GGT- und AP-Werte im Vergleich zu Pi*MM-Individuen.
Fazit Verglichen mit Pi*ZZ-Individuen und Pi*MM-Kontrollen zeigen erwachsene Pi*SZ-Individuen einen intermediären Leberphänotyp, der deutlich milder ist als bei Pi*ZZ-Subjekten.

2.18 Ultraschallgesteuerte Messung der Sarkopenie

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Einleitung Sarkopenie ist nicht nur ein Marker des Ernährungszustandes, sondern zeigt auch einen erheblichen Einfluss auf die Mortalität insbesondere bei Leberzirrhose. Aktueller Standard ist die CT-gesteuerte Messung des M. psoas. Umso wichtiger ist die Vereinfachung der Messung mithilfe des Ultraschalls. Ziel dieser Studie ist es, die Machbarkeit an einem großen Kollektiv zu zeigen und geschlechts-, sowie altersspezifische Unterschiede aufzuzeigen.
Methoden Zwischen März und August 2020 wurde bei 169 ambulanten und stationären Patienten die maximalen Querschnitte des M. psoas und des M. quadratus femoris gemessen. Anhand dieser wurden der PMTH (Psoas muscle thickness height), der TPMAI (totaö psoas muscle area index) und der TMTI (thigh muscle thickness index) berechnet. Geschlechtsspezifische Indices wurden beschrieben und mit Altersgruppen korreliert.
Ergebnisse Die Hälfte der Patienten war männlich (56,8 %), 60 Jahre alt und normal gewichtet (17,96 kg/m2). 45,6 % davon waren hospitalisiert. Der PMTH (Männer: 10,59 mm/m2 bzw. Frauen: 10,54 mm/m2, p = 0,82) und der Femurindex (Männer: 2,21 bzw. Frauen: 2,34, p = 0,26) zeigten keinen geschlechtspezfischen Unterschied. Der TPMAI hingegen ergab einen deutlichen geschlechtsspezifischen Unterschied (280,78 mm2/m2 vs. 247,96 mm2/m2, p = 0,023). Hospitalisierte Patienten zeigten einen größeren Muskelschwund gegenüber ambulanten Patienten (PMTH 9,94 mm/m2 vs 10,99 mm/m2 (p < 0,001), TPMAI 240,92 mm2/m2 vs. 287,55 mm2/m2 (p = 0,003)). Ältere Patienten > 65 Jahren zeigten ebenso einen erhöhten Muskelschwund (p < 0,001)
Diskussion Die ultraschallgesteuerte Messung des M. psoas und des M. quadratus femoris ist praktikabel und könnte in Zukunft die CT-gesteuerte Messung der Sarkopenie ersetzen.

2.19 Machine-learning-based analysis of liver injuries in COVID-19 patients

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Background For several months, global numbers of SARS-CoV-2 infections are rising. The COVID-19 pandemic is a huge challenge for the healthcare system worldwide as well as for the society. Clinicians have to make fast but informed decision. We analyzed a large amount of collected data from the intensive care unit with a machine-learning-assisted algorithm to find essential clinical correlations between parameters and a severe COVID-19 disease.
Methods We used machine-learning algorithms – a gradient boosting model and a neural network autoencoder - to analyze 56 COVID-19 patients from the intensive care unit (ICU) of the University Hospital of Regensburg. The dataset included 136 measurements of high-frequency vital- and respiratory parameters, laboratory measurements and demographical information. Our focus was set on the liver values glutamic-pyruvate transaminase (GPT), Bilirubin, alkaline phosphatase (ALP) and international normalized ratio (INR). We compared these values of 56 COVID-19 patients with the same of 589 non-COVID-19 Patients hospitalized at the ICU at 2019. On this basis, we defined a severity score to classify the liver injury of SARS-CoV-2 infected patients.
The grading of the severity score is defined by the deviation of the measured values to its normal values:

- Severity grade 1: deviation by 1,5-2x of normal value of GPT, Bilirubin and ALP and an INR of 1,15- 1,35
- Severity grade 2: deviation by 2-3x of normal value and 0,5 step of INR
- Severity grade 3: deviation by 3-4x of normal value and 0,5 step of INR
- Severity grade 4: deviation of 4 or more of normal value and 0,5 step of INR

Results 56 SARS-CoV-2 infected patients from the intensive care station were analyzed. The whole patient cohort shows indications of a liver involvement. 8 % show severity grade 1, 19 % severity grade 2 and 10 % severity grade 3. Noteworthy is that 60 % of the COVID-19 patients got the highest severity grade of 4. Furthermore 38 % of the patients with severity grade 4 died.
Conclusions In our study, we show that there is a correlation of a COVID-19 disease and liver involvement. All of the analyzed SARS-CoV-2-infected patients developed massive liver injuries. 60 % of the patients developed the highest severity grade 4 and 38 % of them died. In conclusion, elevated liver values overlap with increased mortality. The challenging task for the future is to find the molecular mechanism of the connection between SARS-CoV-2 and the liver.

2.20 SEAL program - Early detection of liver fibrosis and cirrhosis by screening of the general population

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Question Most patients with liver cirrhosis are detected at a late stage of their disease. In approximately 75 %, diagnosis is based on the development of complications such as ascites or bleeding. At this stage causative treatment interventions are less successful or impossible. Screening for liver health and disease is not included in standard medical check-up programs.

Methods As part of the SEAL program, 16,000 insured persons from a large German health insurance company (AOK) will be offered additional testing of liver enzymes as part of a nationwide primary care program (check-up 35) in two German states, i.e. Rhineland-Palatinate and Saarland. In case of elevated transaminases, the APRI score as a liver fibrosis risk score is calculated. If the APRI score is suggestive for liver disease, patients are referred to a gastroenterologist for further differential diagnostic assessment. Patients suspected to have relevant liver fibrosis are referred to a liver center for further hepatologic workup. Endpoints include data on the epidemiology of elevated liver enzymes, cost-benefit assessments and prevalence of liver fibrosis in a general population.

Results To date, more than 10 000 patients have been enrolled in the SEAL program. The average age of female and male patients was 64 years and 62 years. Of the examined patients, 11 % and 7 % presented with increased ALT and AST values with a maximum of 1.050 U/l and 434 U/l, respectively. In 470 patients a known liver disease was already present at inclusion. Nevertheless, 5 % and 6 % of patients without known liver disease had pathologically elevated ALT and AST, respectively.

Conclusions The establishment of an early diagnosis of liver disease is controversially discussed by professionals and in the literature. The initial data indicates that a relevant group of the population suffers from an increase in transaminases. The SEAL project will provide further data on feasibility, effectiveness and cost-benefit assessment in the German health care system and will contribute substantial evidence to the meaningfulness of such a measure.

2.21 Mesenchymal stromal cells provide hepatic support after extended hepatectomy by modulating thrombospondin-1/TGF-β

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Question Extended partial hepatectomiy carries a high risk of post-surgery liver failure (ePHx), a serious complication for the patient. The pig liver features high anatomical and physiological similarity to the human liver. In a pig model of ePHx, systemic delivery of mesenchymal stromal cells (MSC) supported blood circulation and prevented multiorgan failure. The mechanisms mediating the beneficial MSC effects are largely unknown.

Methods Partial liver resection (70 %) was performed in pigs with and without MSC treatment. Animals were monitored for 24 h post-surgery. Gene expression profiles of lung and liver were obtained by gene array analysis. Subsequent combined bioinformatics analysis predicted organ-independent common molecular targets of MSC action. Predictions were verified in the pig livers and lung, and mechanisms investigated in vitro using cell culture systems.

Results Bioinformatics analysis predicted a novel role for thrombospondin-1 (THBS1) linked to transforming growth factor-beta (TGF-β) and downstream signaling towards providing epithelial plasticity and epithelial-mesenchymal transition (EMT). This was confirmed by the increase in THBS1 and TGF-β in serum and liver associated with impairment of liver function and tissue integrity. MSC attenuated the surgery-induced damage, and decreased THBS1 and TGF-β, as well as epithelial plasticity in both liver and lung. Hence, MSC ameliorated surgery-induced hepatocellular stress and EMT, thus supporting epithelial integrity and facilitating post-resection function and regeneration. Mechanistically, MSC-derived soluble factor(s) inhibited THBS1 secretion from thrombocytes, therewith obviously reducing the availability of active TGF-β and its downstream actions.

Conclusions MSC provide functional support for lung and liver after ePHx, likely by modulation of the thrombospondin-1/TGF-β axis. This might open perspectives to treat multiorgan dysfunction after ePHx by pharmacological interference with thrombospondin-1 secretion and/or action.

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2.22 Preliminary results on simultaneous portal and hepatic vein embolization prior to major hepatectomy

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Introduction Portal vein embolization (PVE) has been standard procedure for hypertrophy of future remnant liver (FRL) prior to major hepatectomies. However, tumor progress might limit surgery if rapid hypertrophy is not assured. Additional hepatic vein embolization (HVE) obstructs outflow pathway, inhibiting arterial flow and induces further damage to embolized liver area and thereby fosters regeneration of contralateral liver.

Methods All consecutive patients undergoing simultaneous right-sided PVE and HVE at our tertiary care centre between 2019 and 2020 were retrospectively included. Underlying malignant identities of patients were cholangiocarcinoma (n = 9), gallbladder cancer (n = 2), colorectal metastases (n = 2), hepatocellular carcinoma (n = 1), Solitary fibrous tumor (n = 1) and melanoma liver metastases (n = 1). FRL and FRL growth rate per day were assessed by volumetry using IntelliSpace Portal station (Philips, Best, The Netherlands) CT viewer software prior and between 13 to 23 days (median 16 days) after PVE and HVE. Further literature based standardised FRL (sFRL), where FRL is calculated in relation to total liver volume based on body surface area (BSA), was calculated with sFRL= FRL/(−794.41+1267.28 × BSA) x 100 with BSA=[weight (kg)×height (cm)/3600].

Results 16 patients (f=6, m=10; age 64.5 years (range 38-82)) were included. Three (19 %) patients received chemotherapy before embolization. Additional embolization of segment IV and middle hepatic vein was conducted in 4 patients (25 %). Mean (±SD) FRL significantly increased from 568.8±148.9 to 832.7±239.6 cm^3 ($p < 0.0001$). The FRL growth rate per

day was 15.1±9.5 cm^3/d. sFRL significantly increased from 33.0±9.0 to 48.0±12.6 % (p < 0.0001).

Conclusion HVE in addition to PVE before major hepatectomy effectively induces FRL growth. HVE should be considered simultaneously to PVE if rapid regeneration is desired.

2.23 Auxiliary two-staged partial resection (ASPIRE) LTx for end-stage liver disease to avoid small-for-size situations

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Question Patients with end-stage liver disease and low MELD scores have limited chances to receive deceased donor livers and therefore require consideration of living liver donation. In this setting donor safety is essential. Donor risks are lower in case of a left liver donation because a larger volume of liver remains in the donor. However, due to lower graft volume, the risk for a small-for-size situation in the recipient may increase. This study aims to prevent small-for-size situations in the recipient using an auxiliary two-staged partial resection liver transplantation of living-donated left liver lobes.

Methods Two patients received a two-stage auxiliary liver transplantation using living-donated left liver lobes after left lateral liver resection. The native extended right liver was removed in a second operation after sufficient hypertrophy of the left liver graft had occurred.

Results No donor developed postoperative complications. In both recipients the graft volume increased by an average of 105 % (329ml to 641ml), from graft-to-body-weight ratio of 0.54 to 1.08 within 11 days after transplantation, so that the remnant native right liver could be removed. No recipient developed small-for-size syndrome, and after a follow-up time of 25 months graft function and overall condition is good in both recipients.

Conclusions Auxiliary two-staged partial resection liver transplantation using living-donor left lobes is technically feasible and can prevent small-for-size situation in adults/adolescents. This new technique can expand the potential living-donor pool, and in selected recipient/donor combinations enables a living liver donation and contributes to increase donor safety.

2.24 Ezrin-polarized circulating tumor cells as a potential biomaker in hepatocellular carcinoma

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Background/Purpose The dynamic polarization of circulating tumor cells (CTCs) plays a major role during metastasis. During this process, tumor cells de- and repolarize. The aim of this study is to evaluate the potential of polarized CTCs (p-CTCs) as a diagnostic marker in patients with hepatocellular carcinoma (HCC).

Methods In this presented study, the CTCs were isolated using Oncoquick®. The immunofluorescence staining of the CTCs followed using Anti-Ezrin-Alexa-Fluor 488® to detect the cytoskeletal and membrane associated protein Ezrin. This study included blood samples of 21 patients with HCC and 18 patients with a non-malignant-liver disease (NMLD).

Results The most common underlying disease was with 33,33 % (n = 7) alcohol abuse followed by non-alcoholic-liver disease (NASH) with 28,57 % (n = 6) and diabetes mellitus type 2 in 23,81 % (n = 5) of the patients. 11 HCC-patients (52,38 %) were suffering from a liver cirrhosis. Most of the patients could be categorized in BCLC-Stadium C (n = 7) followed by BCLC-stadium B (n = 5) and BCLC-stadium A (n = 7). Only 2 patients were BCLC stadium 0. CTCs (1,2 CTCs/ml (0,4-3 CTCs/ml) were detected in 19 HCC patients (90,48 %). The false positivity rate was 16,67 % (n = 3). P-CTCs were found in 15 HCC patients (71,43 %). The false positivity rate was 11,11 % (n = 2). Consequently, the HCC group shows significantly more p-CTCs then the NMLD-Group (p=0.0005). A negative correlation between the tumor size, the BCLC Stadium and the number of p-CTCs (r=-0,309893, rho=-0,216559, p=0,17, p=0,34) was found.

Conclusion The presented results for the proof of polarized CTCs could play an important role in the future study of molecular characterizations of CTCs in the context of liver cancer and offer new ways in the diagnosis of HCC.

2.25 Dynamic of circulating tumor cells in hepatocellular carcinoma under interventional radiological treatment

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Background/Purpose The aim of this study was to investigate the short-term dynamics of circulating tumor cells (CTCs) in patients with hepatocellular carcinoma (HCC) under microwave ablation (MWA) or conventional transarterial chemoembolization (C-TACE). The CTCs shortterm dynamics were compared with the clinical course of the HCC-patients.

Methods Blood samples from 17 patients with HCC who underwent MWA (n = 10) or C-TACE (n = 7) were analyzed. Venous blood was taken before and immediately after the radiological interventions to isolate and quantify CTCs by flow cytometry. CTCs were identified as negative for the marker CD45 and positive for the markers Anti-ASGPR, Anti-CD146 and Anti-CD274 (PD-L1). Patients were followed of up to 2.2 years after the radiological intervention.

Results CTCs were detected in 13 HCC patients (76 %) prior to the radiological interventions. The rate of CTCs were decreased significantly after the intervention in patients treated with MWA (0.4 CTCs/ml of blood, p=0.031). In patients who received C-TACE the CTC-rate decreased nonsignificantly (0.3 CTCs/ml of blood, p=0,3). There were no correlation between the short-term CTC dynamic after the radiological intervention and recurrence rate of HCC.

Conclusion This prelaminary data could confirm the tumoricidal effects of MWA in patients with HCC by significantly decreasing CTCs rate.

2.26 Investigating spontaneous bacterial peritonitis – a novel role of the p53 family in bacteria-host-interaction

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Background Spontaneous bacterial peritonitis (SBP) – a severe complication of liver cirrhosis - is driven by bacterial translocation. Although it is known that bacterial translocation is promoted by immune dysfunctions, increased intestinal permeability and bacterial overgrowth in patients with liver cirrhosis, the detailed mechanism of SBP development is still unknown. With wide-ranging function in immunity and cellular stress response, involvement of the p53 family in liver cirrhosis and SBP is conceivable. Therefore, we studied the regulation of p53 family members and their target function in an intestinal *in vitro* model.

Methods To study intestinal bacteria-host-interaction, we established an intestinal *in vitro* model with HCT-116 epithelial cell line. To mimic bacterial overgrowth, HCT-116 cells were cocultured with *Escherichia coli* (*E. coli*) at different

concentrations for up to 4 hours. Regulation of p53 and p73 were studied via qPCR and Western blot. Additionally, p53 family target functions were analyzed *via* cell death induction upon bacterial stimulation. Using heat inactivation, ultrasonification and filtration, we determined whether contact with viable and intact bacteria is necessary to induce cell death.

Results Coincubation of HCT-116 cells with *E. coli* resulted in a decrease of p53 and p73 protein levels in a time- and dose-dependent manner. Despite reduced levels of p53 family members, a high rate of cell death after *E. coli* stimulation was observed, although apoptosis as an underlying mode of cell death could be excluded. It was further shown that direct contact with viable bacteria was necessary to induce cell death in this *in vitro* model.

Conclusion Bacterial overgrowth as in advanced liver cirrhosis is accompanied with reduced intestinal expression of p53 family members. These reductions are triggered by direct contact with viable bacteria. Thus, this mechanism might contribute to prolonged bacterial replication and SBP development. To antagonize a high bacterial burden, intestinal epithelial cells induce caspase-independent cell death. In summary, these data point out to a new role of p53 regulation in bacterial infection like SBP.

2.27 Epidemiologie von gesicherten bakteriellen Infektionen bei Patienten mit Leberzirrhose in den Jahren von 2010 bis 2019

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Hintergrund Gesicherte bakterielle Infektionen sind häufige Auslöser des akut-auf-chronischen Leberversagens. Das Auftreten von multiresistenten gramnegativen Erregern (MRGN) und Vancomycin-resistenten Enterokokken (VRE) ist mit einer erhöhten Letalität verbunden. Ziel der vorliegenden Studie war die Charakterisierung der bakteriellen Infektionen sowie die Ermittlung der Inzidenz multiresistenter Infektionen bei Patienten mit Leberzirrhose an unserem Zentrum in der letzten Dekade.

Methoden Alle Patienten mit Leberzirrhose, die stationär und ambulant am Uniklinikum Frankfurt von Januar 2010 bis Dezember 2019 in Behandlung waren, wurden eingeschlossen. Von diesen Patienten erfolgte eine Datenbankabfrage aller für bakterielle Infektionen kodierenden ICD-Diagnosen bis zum September 2020 (“Fälle”). Für die resultierenden Fälle wurden alle mikrobiologischen Ergebnisse aus der SWISSLAB Labordatenbank extrahiert.

Ergebnisse Aus 4.063 ambulanten und stationären Zirrhose-Patienten wurden 2.622 stationäre Behandlungsfälle mit bakteriellen Infektionen untersucht. Darunter waren 1.087 Harnwegsinfektionen, 673 Cholangitiden, 489 Atemwegsinfektionen, 399 bakterielle Peritonitiden und 221 Bakteriämien. Insgesamt gelangen bei 862 Patienten (1.643 Fälle) 19.199 Keimnachweise. Davon waren 5.216 aus Urin, 3.037 aus Bronchial/Trachealsekret, 2.193 aus Aszites, 1.045 aus Blut und 618 aus Galle. Mikrobiologisch gesicherte bakterielle Infektionen traten in 137 Patienten (424 Fälle) mit bereits vorbestehender Kolonisation durch MRGN und 86 Patienten (359 Fälle) mit VRE auf. Durch MRGN verursachte Infektionen gab es in 125 Patienten (327 Fälle) und durch VRE verursachte Infektionen in 128 Patienten (284 Fälle). In 28 Patienten (110 Fälle) wurden sowohl MRGN- als auch VRE-Infektionen gesichert. Der Anteil an den Gesamtinfektionen betrug 12,5 % für MRGN-Infektionen (gemittelte jährliche Inzidenz 0,8 %) und 10,8 % für VRE-Infektionen (gemittelte jährliche Inzidenz 0,7 %). Die Inzidenz von VRE-Infektionen nahm während des Beobachtungszeitraums ab, während MRGN-Infektionen anstiegen.

Diskussion Die häufigsten Infektionen waren Harnwegsinfektionen, gefolgt von Cholangitiden, Atemwegsinfektionen, Peritonitiden und Blutstrominfektionen. Multiresistente Infektionen bleiben ein signifikantes klinisches Problem.

2.28 Pruritus Experience in Patients with PBC Treated with Obeticholic Acid Through 6 Years: Patient-Reported Quality of Life

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Question Obeticholic acid (OCA) is approved as second-line treatment for PBC in patients with an incomplete response or intolerance to ursodeoxycholic acid. We evaluated the impact of OCA on patient-reported outcome measures (PROs) of pruritus through 6 years.

Methods POISE was a randomized, double-blind (DB) phase 3 study evaluating OCA 5–10 mg or 10 mg vs placebo over 12 months. A 5-year open-label extension (OLE) followed during which patients received OCA 5–10 mg, or up-titrated to OCA 25 mg. PROs of pruritus were assessed every 3 months: pruritus severity visual analogue scale (VAS; 0 [none]–100 [severe]), 5-D pruritus questionnaire (5-D; 5 [none]–25 [severe]) and PBC-40 itch domain (0 [none]–15 [severe]). Pruritus was managed with concomitant medications, temporary OCA dose interruption or OCA dose reduction.

Results Median (Q1, Q3) scores on the pruritus severity VAS were not significantly different in the OCA 5–10 mg group vs placebo and remained in the mild-to-moderate range in the DB phase. VAS scores in the OCA 10 mg groups were higher than placebo at DB month 3 but similar across groups by DB month 12 (**Figure**). Similar findings were observed during the DB phase for the 5-D and PBC-40 itch median scores. Median VAS (**Figure**), 5-D, and PBC-40 itch scores remained stable through the 5-year OLE phase. All VAS scores remained in the mild-to-moderate category.

Conclusions In POISE, with effective management and correct dose titration, a subset of patients experienced consistently mild pruritus with little impact on quality of life, which was sustained through the OLE phase.

2.29 LIVER STIFFNESS MEASUREMENTS IN EMERGENCY TRIAGE PREDICT INPATIENT HEALTH CARE UTILIZATION

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Background Transient elastography allows non-invasive quantification of liver fibrosis, steatosis or right/global heart failure by liver stiffness measurements (LSM) and controlled attenuation parameter (CAP), respectively. This study aimed to test the feasibility and utility of transient elastography in the emergency department (ED) setting and to investigate whether LSM predicts longer hospitalization and reimbursement for non-elective patients.

Methods LSM and CAP were determined in prospectively recruited consecutive adult patients admitted to the ED of a tertiary referral center. The 9.1 kPa LSM cut-off was used for the diagnosis of significant liver fibrosis (stage ≥ F2), and LSM ≥ 13.0 kPa indicated the presence of cirrhosis. Elastography measurements were correlated with clinical and outcome parameters, including

duration of hospital stay and hospitalization costs based on the German Diagnosis Related Groups (DRG) system.

Results In 200 admitted patients (133 men, age 18 – 97 years), median LSM was 5.5 kPa (2.4 – 69.1 kPa), and median CAP was 252 dB/m (100 – 400 dB/m). In total, 39 patients (19.5 %) presented with LSM suggestive of significant fibrosis, and LSM indicating cirrhosis was detected in 24 patients (12.0 %). Heart failure was diagnosed in 19 patients, which was associated with higher LSM ($p = 0.045$). Patients with LSM ≥ 9.1 kPa were significantly ($p < 0.01$) more likely to require longer hospitalization than those with lower LSM. Patients with LSM ≥ 13.0 kPa generated significantly ($p = 0.001$) higher costs as compared to patients with LSM < 9.1 kPa.

Conclusions Transient elastography represents an easily accessible and anon-invasive screening tool in ED that might help identify patients with advanced liver or cardiovascular diseases in need of increased health care resources.

2.30 Populations-basierte Erfassung des Leberphänotyps bei Alpha1-Antitrypsinmangel

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Hintergrund Der Alpha-1-Antitrypsin Mangel (AATM) ist eine genetische Erkrankung, die sowohl Lunge als auch Leber betrifft und einen tödlichen Verlauf nehmen kann. Bis heute werden viele Patienten nicht diagnostiziert. Aus diesem Grund stellt die populationsbasierte UK Biobank eine Möglichkeit dar, die Relevanz der wichtigsten AATM-Allele "Pi*Z" und "Pi*S" zu analysieren.

Methoden In der UK Biobank wurde der Leberphänotyp von Pi*SS (homozygote Pi*S-Präsenz), Pi*MZ (heterozygote Pi*Z-Präsenz), Pi*SZ (ein Pi*S und ein Pi*Z-Allel), Pi*ZZ Individuen und Pi*S/Pi*Z-Nicht-Trägern untersucht. Analysiert wurden hierbei 444.642 Teilnehmer. Untersucht wurden Serumparameter und ICD10-Codes der Probanden. Die Auswertungen wurden auf Alter, Geschlecht, BMI, Alkoholkonsum und Diabetes mellitus korrigiert.

Ergebnis In der Kohorte befanden sich 138 Pi*ZZ (Frequenz 1:3499), 866 Pi*SZ (1:558), 1014 Pi*SS (1:476) und 17017 Pi*MZ Probanden (1:28). Die Transaminasen, repräsentiert durch ALT- und AST-Werte, waren in allen AATM-Genotypen signifikant höher als bei Nicht-Trägern. Pi*ZZ-Personen wiesen die höchsten AST-Werte auf. ALP war bei Pi*MZ- und Pi*SZ-Teilnehmern im Vergleich zu Nicht-Trägern signifikant erhöht. Die ICD10-Diagnose "Leberfibrose/Zirrhose" war bei Pi*ZZ-Personen fast 20-fach häufiger als bei Nicht-Trägern, aber auch deutlich angereichert in Pi*SZ- und mäßig bei Pi*MZ-Probanden. Pi*SZ- und Pi*ZZ-Individuen hatten 7 bzw. 45fach erhöhtes Risiko für die Diagnose von primärem Leberkrebs. Darüber hinaus waren sie prädisponiert für Neoplasien von Leber- und/oder Gallengängen. Männliches Geschlecht, Alter >50, Diabetes mellitus und Adipositas waren assoziiert mit einer signifikanten Leberfibrose.

Fazit Diese populationsbasierte Analyse definiert den Leberphänotyp bei den wichtigsten AATM Genotypen. Diese Daten sollten daher das evidenzbasierte Monitoring dieser Menschen ermöglichen.

2.31 Blood cyclic guanosine monophosphate levels as potential marker of portal hypertension in patients with liver cirrhosis

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Question Despite intensive research reliable blood-derived parameters to detect portal hypertension in patients with liver cirrhosis are lacking. As dysregulation of the nitric oxide - cyclic guanosine monophosphate (NO – cGMP) pathway is considered a major factor in the pathogenesis of portal hypertension, the aim of our study was to investigate blood cGMP levels in different stages of chronic liver disease and to evaluate cGMP as potential biomarker of portal hypertension.

Methods cGMP levels were analyzed in blood of patients with liver cirrhosis and clinically significant portal hypertension (n = 32), patients with liver cirrhosis without clinically significant portal hypertension (n = 21), patients with chronic liver disease without cirrhosis (n = 11) and healthy controls (n = 8). cGMP levels were evaluated as biomarker of portal hypertension by ROC-analyses in comparison to the Lok-index as established score to predict portal hypertension.

Results Blood cGMP was significantly elevated in liver cirrhosis patients who had clinically significant portal hypertension compared to liver cirrhosis patients without portal hypertension (90.1 ±46.8 pmol/ml vs. 50.1 ±46.4 pmol/l, $p < 0.001$). In contrast, there was no significant difference in blood cGMP between patients with liver cirrhosis without portal hypertension and patients without liver cirrhosis/fibrosis ($p = 0.347$). ROC-analyses revealed a high diagnostic value of blood cGMP for the prediction of portal hypertension (AUC 0.925 [0.827-0.999]) that was superior to the Lok-index (0.737 [0.568-0.907]).

Conclusions Blood cGMP may be a valuable biomarker of cirrhotic portal hypertension in clinical practice that invites further investigation.

2.32 Biomarkers for Diagnosis of Sepsis in Patients with Liver Cirrhosis

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Background Patients with liver cirrhosis show immune dysfunction and bacterial translocation. The overall mortality rate of septic shock remains particularly high in cirrhotic patients, ranging from 60 % to 100 %. It is difficult to predict sepsis in patients with cirrhosis because cirrhosis itself can lead to clinical presentation of sepsis. The levels of C-reactive protein and Procalcitonin in patients with liver cirrhosis correlate moderately with the severity of infection. Thus, new diagnostic tools are needed.

Methods The aim of the study was to evaluate the serum levels of Presepsin, Calprotectin and S100A12 protein in patients with liver cirrhosis and sepsis. The study was initiated in August 2018 and is still ongoing. We took blood samples from patients with different causes of sepsis and performed Enzyme-linked Immunosorbent Assays (ELISA). Blood samples were taken within 24h after admission on the intensive care unit at the University Hospital Regensburg, Germany. Until now we have 79 blood samples from patients with different causes of sepsis archived. Besides other comorbidities, liver cirrhosis was present in almost 50 % of the cohort (31 patients). We compared our values with the standard values of Presepsin, Calprotectin and S100A12. Furthermore, we compared the values of the three blood markers in patients with and without liver cirrhosis.

Results Patients with sepsis and liver cirrhosis in our cohort had significantly lower Calprotectin, S100A12 and Presepsin values compared to septic patients without cirrhosis. Concerning Calprotectin only 38 % (12 Patients) of patients with liver cirrhosis and sepsis had higher levels of serum calprotectin compared to the normal values. The S100A12 protein showed also weak abilities to diagnose sepsis in patients with liver cirrhosis. All the values were in the normal range.

Regarding Presepsin, 99 % of septic patients (with and without liver cirrhosis) had higher serum levels and all septic patients with liver cirrhosis had higher levels of serum presepsin compared to the normal values.

Conclusion In our cohort Calprotectin and the S100A12 protein were less suitable to diagnose sepsis in patients with liver cirrhosis. However, Presepsin was an excellent marker for the detection of sepsis not only in patients with liver cirrhosis but also in patients without cirrhosis.

Thus, Presepsin could be beneficial in daily clinical practice in diagnosis of sepsis, even in patients with liver cirrhosis.

2.33 Hospital mortality and current developments of liver transplantation in Germany: A systematic 10-year analysis

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Background Both internationally and in Germany, liver transplantation (LT) has undergone a dynamic development in recent decades. This has included a complete change in the allocation system with the introduction of the MELD-system, but also challenges such as those arising from the irregularities at four German liver transplant centers exposed in 2012. In addition, there is an ongoing discussion in Germany about the total number of centers at which LT is carried out and the Joint Federal Committee (G-BA) has recently reinforced the guidelines regarding minimum transplantation numbers. A systematic evaluation of existing data sets and their careful interpretation can support a rational discussion aiming at optimizing framework conditions of LT.

Methods We used standardized hospital discharge data (diagnosis-related groups and German operations and procedure key codes) to analyze hospital mortality after LT in Germany between 2008 and 2017. Data were provided by the Federal Statistical Office of Germany.

Result A total of n = 9254 LT procedures performed between 2008 and 2017 were included into the present analysis. Annual LT frequency decreased by 24.09 % from 2008 (n = 984) to 2017 (n = 747). The average hospital mortality rate of all LT procedures was 14.3 % and showed a significant improvement during the observation period (2008: 15.8 %, 2017: 11.0 %). Hospital mortality significantly correlated with recipient age (< 40 years: 9.4 %, ≥40 years: 12.9 %, ≥50years: 15.5 %, ≥60 years: 17.4 %) but did not further increase in patients ≥70 years (16.5 %). We identified the underlying liver disease etiology, the need for relaparotomy as well as prolonged mechanical ventilation as further factors associated with an increased hospital mortality. In addition, hospital mortality significantly differed between German federal states ranging from 9.6 % to 24.1 %. Finally, transplantation centers that performed less than 20 LT annually had a significantly higher mortality rate than centers that performed ≥20 LT yearly.

Conclusion LT-related hospital mortality has fallen steadily over the last years, and LT is a safe therapy even in well-selected elderly recipients. Our study identified a number of variables associated with increased mortality. Against the background that our study considers parameters of the current G-BA guideline on minimum numbers of LT, these data provide a scientific basis for the current discussion on the reorganization of LT in Germany.

2.34 Biliary injury in patients with severe COVID-19 is most likely not caused by direct viral damage

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Introduction Infection with the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has developed into a pandemic with millions of infected people worldwide. Although an elevation of liver enzymes has often been observed especially in severe cases, we were able to document the first case of secondary sclerosing cholangitis (SSC) after coronavirus disease 2019 (COVID-19). The correlation between hepatobiliary injury and the infection is not fully understood yet.

Methods A patient was diagnosed with SSC by MRCP, ERCP and liver histopathology. COVID-19 RNA was detected by PCR-analysis of the patient's nasal/throat swabs, tracheal fluid and stool samples. Post-mortem tissue as well as swab-material of liver, bile ducts, gallbladder, duodenum and lung samples of people deceased after SARS-CoV-2 were analyzed for COVID-19 viral load.

Results We are describing the world's first case of secondary sclerosing cholangitis (SSC) following severe acute respiratory distress syndrome due to COVID-19 confirmed by liver imaging and histopathology. Most likely

causes of SSC in this patient include direct viral damage to the biliary epithelium, drug toxicity and immune-mediated- or hypoxia-associated cell injury. To investigate the role of direct viral damage to the biliary epithelium, we were measuring COVID-19 levels in the patient"s stool sample as well as COVID-19 levels in bile and duodenum of five patients deceased after severe COVID-19 infection and in one patient undergoing cholecystectomy and ERC for acute cholecystitis with choledocholithiasis during infection with SARS-CoV-2.

Our index patient tested positive for COVID-19 RNA in a stool sample. However, we were not able to find viral RNA in biliary tissue or bile samples from deceased or cholecystectomized SARS-CoV-2 positive patients, so that we were not able to find direct proof for viral replication of COVID-19 in biliary cells *in vivo*. While direct biliary damage in our patient with SSC due to COVID-19 cannot be ruled out, histopathological findings of the liver are most consistent with SSC in the critically ill caused by hypoxia.

Conclusion In conclusion, we investigated the first case of SSC in a patient after severe COVID-19 infection emphasizing the importance of follow-up care to recovered patients to determine the full complexity of its long-term effects.

2.35 Modifikation des CLIF-C ACLF-Scores für die Prognoseabschätzung bei Schutzintubation und Lungenversagen.

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Hintergrund und Ziele Das akut-auf-chronische Leberversagen (ACLF) ist durch eine akute Dekompensation, Mehrorganversagen und eine hohe Kurzzeit-Mortalität charakterisiert. V.a. bei beatmeten Patienten mit ACLF ist eine adäquate Risikostratifikation essentiell. In dieser Untersuchung wird die Vorhersagekraft des CLIF-C ACLF Scores untersucht und dieser Score für das Vorliegen von mechanischer Beatmung und Lungenversagen angepasst.

Patienten und Methoden In dieser retrospektiven Untersuchung wurden 498 intensivpflichtige Patienten mit Leberzirrhose eingeschlossen. Ein ACLF wurde gemäß EASL-CLIF Kriterien definiert. Wir führten ein 1:1:1 Propensity Score Matching durch, als Kovariablen wurden hierzu der CLIF-C ACLF/AD Score und das Geschlecht verwendet. Die prädiktive Performance von etablierten Scores wurde mittels ROC Analysen evaluiert.

Ergebnisse Insgesamt zeigten 216 Patienten (43.4 %) ein ACLF bei Aufnahme. Mechanische Beatmung (MV) und Lungenversagen (RF) konnten in der 1:1:1 gematchten Kohorte (MV:RF:noMV, n = 147) als Risikofaktoren für eine signifikant höhere Kurzeit-Mortalität identifiziert werden (28-Tage-Mortalität: 83.7 % vs. 67.3 % vs. 38.8 %). In der ROC Analyse zeigte der CLIF-C ACLF Score die beste Prädiktion der Kurzzeit-Mortalität, auch in allen ACLF Organversagens-Subgruppen mit Ausnahme von ACLF-RF (AUROC 0,49) und ACLF-MV (AUROC 0,68). Es wurde eine Kalibrierungsvariable (CV) in den CLIF-C ACLF Score eingerechnet, die RF/MV abbildet (CV = 1 für RF; 0,5 für MV; -0,1 für noMV/noRF). Der angepasste Score JAM = CLIF-C ACLF + (20*CV) zeigt eine signifikant verbesserte prädiktive Performance für die 28-Tage-Mortalität (AUROC 0,87 vs. 0,81, n = 498).

Schlussfolgerung Die mechanische Beatmung und das Lungenversagen im ACLF führt unabhängig vom ALCF Schweregrad zu einer erhöhten Kurzzeit-Mortalität, beide sind jedoch nicht adäquat im CLIF-C ACLF Score abgebildet. Der hier vorgeschlagene JAM-Score verbessert die Vorhersagekraft des Kurzzeit-Überlebens deutlich.

2.36 Is there a too old in liver donors? An age stratified analysis of elderly liver donors above 65

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Question Scarcity of available organs has led to an increase in accepting marginal grafts. While it has been established that donor age above 65 years is an independent risk factor for graft loss, further analyses within this cohort are still limited. Aim of this analysis was to investigate the influence of stratified donor age on 1-year outcome after orthotopic liver transplantations (oLT).

Methods A retrospective analysis including all oLT patients at the University Hospital Münster between 2006 and 2017 was conducted. Eligible patients were stratified for donor age: ≥ 65-69 vs. ≥ 70 years of age. Baseline donor and recipient characteristics were compared, and primary endpoint was 1-year patient survival. Secondary endpoint was overall 1-year graft survival.

Results A total of 350 oLT patients were identified, of which 47 received a graft ≥ 65-69 and 35 a graft ≥ 70 years of age. Both groups revealed a comparable recipient age while age difference between donor and recipient was 10.4 ± 8.5 (donor ≥ 65-69) and 18.1 ±12.4 years (donor ≥ 70, p = 0.002). Baseline characteristics (indication for oLT, ischemia times, MELD score and donor risk index) were similar. One-year patient survival was 76.6 % (≥65 - 69) and 68.6 % (≥ 70, *p*= 0.40). Overall 1-year graft survival was 72.3 % (donor ≥65 - 69) and 65.7 % (donor ≥ 70, *p*= 0.50).

Conclusion A slightly higher but not significant increase in graft loss and lower patient survival was found for grafts of donors older than 70 years of age compared to those between 65 and 69. While both groups revealed acceptable survival rates, these results demonstrate that donor age above 70 years is associated with an increased risk for inferior outcome for patient and graft survival within the first year after oLT.

2.37 Hepatic sarcoidosis

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Sarcoidosis is a systemic disease of unknown etiology involving multiple organs. Main manifestations are biliary lymphadenopathy, pulmonary densification as well as skin, joint and eye involvement. However, liver involvement was estimated to occur in up to 60 % of all patient. As many patients experience only minor symptoms, a high number of undiagnosed cases must be assumed. In order to identify patients with hepatic sarcoidosis a throughout characterization of these patients and their course of disease is necessary. However, the currently available evidence on hepatic sarcoidosis were collected from the 1970s when treatment of liver disease was clearly different form today"s standards. Thus, we collected 43 patients from 4 German centers to evaluate current treatment standards and course of disease. All our patients had liver biopsy with histologically proven granulomatous hepatitis to confirm sarcoidosis. Detailed

characterization of these patients showed an overall benign course of disease. Treatment of these patients was very diverse with glucocorticoids for 1 year in 57 %, 5-10 years in 17 %, and permanently in 20 % of patients. Other treatments included DMARD in 23 % of patients (40 % azathioprine, 23 % MTX, 5 % hydroxychloroquine, 5 % MMF, and 5 % cyclophosphamide). Biologicals were administered to 7 % of all patients. Despite these very diverse treatments patients generally showed only slow progress. None of our patients received liver transplantation, or suffered from significant liver related morbidity, or mortality.

Overall, our cohort indicated that morbidity and mortality of sarcoidosis as a systemic disease is mostly not dependent on hepatic manifestation as progression is slow and symptoms generally mild.

2.38 Variceal Bleeding has Increased Mortality Compared to Non-Variceal Bleeding only in Males.

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Question Gastrointestinal bleedings (GIB) are frequent in cirrhotic patients and lead to high morbidity and mortality. Lately, there have been conflicting reports on the role of and bleeding type (variceal bleeding [VB] and non-variceal bleeding [NVB]).

This study investigated predictors of mortality in patients with VB and NVB with relationship to sex differences.

Methods 271 patients with suspected upper GIB who underwent endoscopy were included. Patients were followed up at one week, six months and one year after admission. Uni– and multivariate logistic or Cox regression analyses investigated correlations of predictive factors and clinical outcomes. Propensity score matching was performed to control for severity of disease and compare groups for sex and bleeding type.

Results 42 patients were excluded (cirrhosis or bleeding not confirmed). The remaining patients were classified by bleeding type into patients with VB (n = 115) or NVB (n = 156). Males (n = 155) had a higher mortality in VB than in NVB, while in females (n = 116) mortality was similar in the two bleeding types. This was confirmed after matching in males (n = 116) and females (n = 82). Further independent predictors of mortality in males were MELD at baseline, BUN, ALT, while in females age, leukocytes, MELD, history of ascites and hepatic encephalopathy.

Conclusion This study shows that VB has higher mortality in males compared to NVB, while in females the type of GIB does not impact outcome.

2.39 Stool and sputum microbiome during quinolone prophylaxis of spontaneous bacterial peritonitis

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Introduction Quinolone prophylaxis is recommended for patients with advanced cirrhosis at high risk of spontaneous bacterial peritonitis (SBP) or with prior SBP. Yet, the impact of long-term antibiotic prophylaxis on the microbiome of these patients is poorly characterized.

Methods Patients with liver cirrhosis receiving long-term quinolone prophylaxis to prevent SBP were prospectively included and sputum and stool samples were obtained at baseline, one, four and twelve weeks thereafter. Both bacterial DNA and RNA were assessed with 16S rRNA sequencing. Relative abundance, alpha and beta diversity were calculated and correlated with clinical outcome.

Results Overall, 35 stool and 19 sputum samples were obtained from 11 patients. Two patients died (day 9 and 12) all others were followed for 180 days. Reduction of Shannon diversity and bacterial richness was insignificant after initiation of quinolone prophylaxis ($p > 0.05$). Gut microbiota were significantly different between patients ($p < 0.001$) but non-significantly altered between the different time points before and after initiation of antibiotic prophylaxis ($p > 0.05$). A high relative abundance of *Enterobacteriaceae* >20 % during quinolone prophylaxis was found in three patients. Specific clinical scenarios (development of secondary infections during antibiotic prophylaxis or the detection of multidrug-resistant *Enterobacteriaceae*) characterized these patients. Sputum microbiota were not significantly altered in individuals during prophylaxis.

Conclusion Inter-individual differences in alpha and beta diversity of gut microbiota were high at baseline, yet quinolone prophylaxis had only a moderate impact on gut microbiota diversity. High relative abundances of *Enterobacteriaceae* during follow-up might indicate failure of or non-adherence to quinolone prophylaxis. Future studies are needed to further investigate this phenomenon and to tailor individual prophylactic strategies in these patients.

2.40 Liver cirrhosis, age, and bilirubin could be prognostic for mortality in pancreatitis

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Background and Aim Alcohol abuse and subsequent alcohol-related liver disease and pancreatitis are still among the most common hepatological diseases for hospital admittance in industrialized societies. It remains unclear why excessive alcohol use leads to either liver cirrhosis (LC) in some patients or an acute/chronic pancreatitis in others or both. In our study we analyzed patient data from a single-center retrospectively to elucidate potential risk factors and/or characteristics, trying to understand better, which patients with alcoholic abuse disorder develop pancreatitis alone or in combination with liver cirrhosis.

Method In a retrospective single-center study data of 625 patients was analyzed, who were admitted to the University Hospital Magdeburg because of acute or chronic pancreatitis or developed it during their stay between 2013 and 2017. We further analyzed which of these patients had LC or had been diagnosed with it during their hospital stay. Epidemiological data, serum parameters and, where available, liver steatosis were analyzed for patients with either alcohol-related or biliary-obstructive pancreatitis.

Results After exclusion of patients with incomplete data or other etiologies for pancreatitis 403 patients were included in the study. 279 were male and 124 were female; 198 suffered from an alcohol-related pancreatitis and 205 from biliary pancreatitis. Relatively more men suffered from alcoholic-related than biliary pancreatitis and patients with biliary pancreatitis were older. Prevalence of steatosis and LC and mortality did not differ between etiologies. For alcohol-related pancreatitis higher serum levels of AST, AP, and bilirubin were predictive of LC. In patients with biliary pancreatitis lower serum ALT concentrations and higher creatinine were associated with LC. Risk factors for mortality were analyzed combined for both etiologies, as only 21 patients deceased in the

whole cohort. Presence of LC, older age, and higher serum concentrations of bilirubin, AP, and creatinine were associated with mortality.

Conclusion Pancreatitis related to alcohol or biliary cause differ in serum derived factors and gender distribution but not in clinical presentation. LC, age, bilirubin, creatinine, and AP could be prognostic factors for outcome in pancreatitis independent of etiology.

2.41 Outcome after liver resection for hepatic hemangiosarcoma

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Question Primary hepatic angiosarcoma (PHA) is a malignant and very uncommon mesenchymal tumor that develops from endothelial cells and constitutes to about only 2 % of all primary liver tumors. Radical resection is presently considered the only potentially curative treatment alternative. The purpose of this study is to evaluate postoperative morbidity and overall survival (OS) of patients who underwent hepatic resection for PHA in our clinic.

Methods We retrospectively analyzed clinicopathological data of all consecutive patients who underwent liver resection in curative intent for PHA between 2012 and 2020 in our center.

Results During the study period six patients underwent liver resection for PHA in curative intent in our center: 83 % (5/6) of patients were female, and 17 % (1/6) were male, with a median age of 64.2 years (range 44 - 81). 1/6 patients had an ASA 3 and 5/6 an ASA 2 status, respectively. Two patients went through non-anatomical hepatic resection; two patients had a right hemihepatectomy; one patient underwent left lateral resection, and one a trisegmentectomy. The postoperative morbidity was high with 83 % (5/6): Three patients suffered from minor postoperative complications (Clavien-Dindo grades 1 and 2), one patient from major postoperative complications (Clavien-Dindo grade 4A) and one patient died after the procedure (Clavien-Dindo 5). Median overall survival was 25.15 months.

Conclusion Although resection prevails as the best treatment option for PHA, postoperative morbidity is frequent and the overall prognosis remains poor. Better diagnostic modalities and adjuvant treatment options are needed to improve the prognosis of patients suffering from PHA.

2.42 Beneficial Effects of Nuclear Magnetic Resonance Therapy on Liver Regeneration

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Question Liver resection is often the only feasible therapy option in treating benign and malignant diseases of the liver. Thereby, curative surgery is depending on the removal of the adequate amount of parenchyma, with the challenge of leaving a sufficient amount of functional liver tissue in situ. Until now, nuclear magnetic resonance therapy (NRMT) is utilized for osteoarthritis (OA) or osteoporosis treatment with promising results leading to improvement of patient"s function. In vitro experiments confirmed elevated proliferation rates for human chondrocytes and osteoblasts under culture conditions. To potentially improve liver regeneration after parenchyma resection, the aim of the present study was to investigate effects of NRMT on liver cells in an animal model of critical liver resection.

Methods Rats (n = 54) were randomly assigned to three different groups: 1. sham group without NMRT; 2. treatment with a frequency normally used for OA; 3. treatment with a frequency normally used for osteoporosis and bone"s circulatory disorder with n = 18 animals, respectively. All rats received a 70 % liver resection. Rats were sacrificed after 4, 7 or 14 days and livers were histologically analyzed for liver regeneration (for mitosis rate BrdU and Ki 67; for angiogenesis VEGF) and injury (silver- and HE-staining and immunohistochemical determination of anti-vimentin).

Results No negative/injuring effects of NMRT on rat liver tissue were found. Moreover, treatment with different frequencies lead to altered mitosis rates and time of liver regeneration. Treatment with NMRT revealed a better cell proliferation with significantly higher mitosis rate and significantly smaller cells indicating a terminated regeneration process.

Conclusions An accelerated regeneration capacity could ameliorate the options in liver surgery and postoperative outcome. Further studies are needed to not only confirm but also clarify the beneficial effect of NMRT on parenchyma of the liver.

2.43 The multidisciplinary approach to the complex treatment for non-cirrhotic portal hypertension

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Non-cirrhotic portal vein thrombosis (PVT) in patients with antiphospholipid syndrome (APS) is a rare complication and the management has to be determined individually based on the extent and severity of the presentation. We report on a 37-year-old male patient with non-cirrhotic chronic PVT related to a severe thrombophilia, comprising APS, antithrombin-, factor V- and factor X-deficiency. Three years after the initial diagnosis of non-cirrhotic PVT, the patient presented with severe hemorrhagic shock related to acute bleeding from esophageal varices, requiring an emergency transjugular intrahepatic portosystemic stent shunt (TIPSS). TIPSS required revision after a recurrent bleeding episode due to insufficient reduction of the portal pressure. Embolization of the massively extended V. coronaria ventriculi led to the regression of esophageal varices but resulted simultaneously in a left-sided portal hypertension (LSPH) with development of stomach wall and perisplenic varices. After a third episode of acute esophageal varices bleeding, the decision to add on a distal splenorenal shunt (Warren shunt) to reduce the LSPH was made. Despite anticoagulation with low molecular weight heparin and antithrombin substitution, endoluminal thrombosis led to a complete Warren shunt occlusion, aggravating the severe splenomegaly and pancytopenia. Finally, a partial spleen embolization (PSE) was performed. In the postinterventional course, leukocyte and platelet counts increased rapidly and the patient showed no further bleeding episodes. Overall, this complex course demonstrates the need for individual assessment of multimodal treatment options in non-cirrhotic portal hypertension. This young patient required triple modality porto-systemic pressure reduction (TIPSS, Warren shunt, PSE) and involved finely balanced anticoagulation and bleeding control.

2.44 Liver Resection for Non-colorectal, Non-neuroendocrine Metastases

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Question Hepatic resection has become a standardized, well-established surgical procedure with substantially improved survival for patients with colorectal and neuroendocrine liver metastases. However, the value of this procedure in non-colorectal and non-neuroendocrine (NCRNNE) metastases remains unclear. This study aims to analyze patients undergoing liver surgery for NCRNNE metastases.

Methods All patients who underwent liver resection for NCRNNE metastases between 2012 and 2017 were included in our study. The outcome of the patients was analyzed according to the primary tumor, histopathology, type of therapy and demographics.

Results Thirty-one patients (16 males, 15 females), with an average age of 54.4 (20-80) years, underwent liver resection with curative intent during the observation period. The most common primary tumors were pancreatic adeno carcinoma (4), renal cell carcinoma (3), esophageal cancer (3), gastrointestinal stromal tumor (3), melanoma (3) and thyroid cancer (3).

Among them, twelve patients were suffering from synchronous liver metastases (38.7 %). [HH1] Microscopic margin-free resection could be achieved in 90.3 % of the patients.

The overall survival rates for 1 year and 3 years were 61.3 % and 45.2 %, respectively.

Conclusion Liver resection should be considered as a curative procedure for patients with metastatic NCRNNE. Further studies are needed to identify the factors allowing improved survival, especially with additive chemotherapeutic regimen.

2.45 De novo Crohn disease after liver transplantation: Case Report and Review of the literature

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Although, inflammatory bowel disease (IBD) is an important differential diagnosis yet in patients with immunosuppressive treatment, especially calcineurin-inhibitors, only very few patients are reported. We present a patient with de novo Crohn disease (CD) after liver transplantation. The patient was diagnosed under immunosuppressive treatment after antecedent liver transplantation due to polycystic liver and kidney disease. Our patient suffered from abdominal pain, chronic diarrhea and episodes of fever and shivering for months until the diagnosis of CD was made and intensification of immunosuppressive treatment led to improvement of symptoms. Treatment with additional or increased corticosteroids is feasible and may lead to rapid response. In order to foster the discussion on those mechanisms we furthermore bring our case in perspective with the available literature.

Poster Visit Session III Metabolism (incl. NAFLD)
Friday, January 29, 2021, 4:40 pm – 5:25 pm, Poster Session Virtual Venue

3.4 Characterization of the liver scavenger cell proteome in mouse and rat

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Introduction The liver is characterized by a functional and metabolic organization which is established through specialized hepatocyte subpopulations. For instance, depending on their localization within the acinus, ammonia metabolising functions of these subpopulations differ strikingly. While periportal hepatocytes contribute to ammonia detoxification through the urea cycle, perivenous scavenger cells prevent ammonia spill over into the circulation by binding NH4+ ions to glutamate via glutamine synthetase (GS). Despite intense research efforts, it is still unclear whether populations of scavenger cells is homogeneous or composed by subpopulations.

Methods By means of FACS sorting and mass spectrometry, proteome profiles of scavenger cells was compared to those of perivenous hepatocytes in mouse and rat. Newly identified scavenger cell markers were further investigated by immunofluorescence analyses. To investigate whether the distribution of ammonia metabolism-related proteins is altered in liver diseases, liver sections of patients with hepatocellular carcinoma (HCC) and liver cirrhosis were studied by immunofluorescence analyses.

Results Besides established markers, novel scavenger cell-enriched proteins like the basal transcription factor 3 (BTF3) or the heat-shock protein 25 (HSP25) were identified in mouse and rat. Interestingly, BTF3 and HSP25 were not homogeneously distributed among scavenger cells, but high amount of these proteins were found in particular subsets of cells. Protein levels and distributions of investigated proteins were altered in HCC and cirrhotic tissue compared to healthy livers. For instance, levels of BTF3 and HSP27, the human HSP25 homologue, were enhanced in HCC compared to healthy livers. Our data further showed that the colocalization of GS with BTF3, HSP27, glutamate transporter 1 (GLT1) and ammonium transporter Rh type B (RHBG) was nearly absent in HCC tissue.

Conclusions Our data suggest that the scavenger cell proteome is highly diverse which is indicative for a yet unknown functional heterogeneity of these cells. It was further shown that levels and distribution of scavenger cell-enriched proteins are altered in HCC *versus* normal livers. The presented data open up new perspectives and warrant future research on the functional and metabolic zonation of the liver.

This work was supported by Deutsche Forschungsgemeinschaft through SFB974 - Communication and System Relevance in Liver Injury and Regeneration.

3.5 THE LIVER COPPER STATUS ALTERS THE DEVELOPMENT OF STEATOSIS IN MICE

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Question The association between copper (Cu) misbalance and liver steatosis has been reported in experimental models and in human disease. Inactivation of the Cu transporter ATP7B causes Cu accumulation in the liver and Wilson Disease (WD). WD patients have low Cu in the serum, high Cu in the liver, and often develop liver steatosis. In nonalcoholic steatohepatitis (NASH), patients also have low serum Cu and show inverse correlation between the lipid and Cu content in the liver. The contribution of Cu to the development of liver steatosis is not well understood. We tested the effect of a high-fat-high-sugar diet (HFHSD) in C57BL/6J Atp7b-/-knockout mice (WD model) and compared it to C57BL/6J (wt). Further, we tested if the HFHSD in reverse influences the WD phenotype.

Methods C57BL/6J (wt) and C57BL/6J Atp7b-/- mice were fed HFHSD or normal chow (NC) to 16 weeks of age. All four groups were evaluated for their fat mass per weight (NMR spectrometry), liver enzymes, lipid profile, copper levels

(by atomic absorption spectrometry), and mitochondrial function (measuring NADP+/NADPH ratios). Additionally, proteomics analyses of male livers were performed at 16 weeks to compare effected pathways.

Results Atp7b-/- mice accumulate less fat on HFHSD compared to wt mice; the effect is more pronounced in males. Furthermore, the development of the WD phenotype is less severe in Atp7b-/- mice fed the HFHSD. Liver enzymes are less elevated in Atp7b-/- mice on HFHSD compared to NC, and lower than in wt mice on HFHSD. Cu levels are lower in Atp7b-/- mice fed the HFHSD at 16 weeks, but not at 10 weeks of age, suggesting a potential metabolic utilization of Cu in HFHSD. Proteomics analyzes reveal changes affecting the cholesterol and bile acid biosynthesis for the HFHSD groups and an improved proteomics profile.

Conclusions Our results suggest an influence of Cu homeostasis on the development of steatosis. We were surprisingly able to show that elevated Cu levels in the liver (WD model) lead to a decreased accumulation of fat compared to the wildtype animals when fed a HFHSD. Our data also indicate an effect of the HFHSD on the WD phenotype, when comparing the ATP7B-/- knockout animals fed HFHSD vs. NC. Strikingly, we show a decrease in Cu levels at the age of 16 weeks in ATP7B-/- HFHSD mice, suggesting a Cu usage in a HFHS environment. In conclusion, we show an interaction between copper homeostasis and steatosis development in a WD mouse model.

3.6 Macrophage-specific iPla$_2$β deletion worsens hepatic inflammation in MCD-induced NASH by increasing M1/M2 activation.

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Background Polymorphisms of iPLA$_2$β are associated with an increase of serum C-reactive protein suggesting its role in inflammation. It is known that iPla$_2$β regulates monocyte differentiation and monocyte migration to sites of inflammation, however, its role in NASH is still elusive. We previously reported that female iPla$_2$β-null mice fed with methionine- and choline-deficient diet (MCDD) displayed opposing phenotypes; on one hand attenuation of liver enzymes and liver fatty acids but on the other hand exacerbation of liver fibrosis (BBA 2019, 1864, 677). Here, we examined whether macrophage-specific iPla$_2$β deletion has any effects on NASH induced by MCDD.

Methods Macrophage-specific (lysM-Cre) iPla$_2$β-deficient (KO) mice with exon 6-8 deletion were generated. Female WT or iPla$_2$β$^{flox/flox}$ were used as controls. KO mice were fed with chow or MCD for 3.5 weeks. Livers were harvested for RT-PCR analysis. Blood and liver lipids were analyzed.

Results MCDD-fed control mice induced hepatic steatosis as observed by an increase of liver lipids. MCDD-fed KO mice showed a further increase of hepatic triglycerides (TG) without altering serum TG, phospholipids, and free fatty acid levels. MCD feeding caused a severe reduction of body weights and an increase of normalized liver weights in both controls and KO. Under MCD feeding, KO showed a further elevation of hepatic inflammation as observed by mRNA expression of F4/80, Ly6C, CD68, CD11b, MCP1, CCL3, VCAM1, and ICAM1 as well as M1 markers TNF-α and IL-6. In control mice, MCDD feeding increased mRNA expression of M2 markers including arginase-1 and chitinase-like 3, and interestingly, these markers were further increased by the deficiency. Moreover, iPla$_2$β deficiency further increased hepatic fibrosis as measured by collagen1-alpha, plasminogen activator inhibitor-1, and tissue inhibitor of metalloproteinases-1 mRNA expression.

Conclusions Under MCDD, iPla$_2$β-null mice showed an increase of liver fibrosis without altering liver inflammation, while macrophage-specific iPla$_2$β deletion enhanced liver fibrosis and inflammation. Thus, iPla$_2$β deficiency in other cell types may play a protective role in global deficiency. In addition to M1, macrophage iPla$_2$β deficiency increased M2 phenotypes rendering susceptibility to MCDD-induced lean NASH. Our study presents novel findings revealing a protective role of iPla$_2$β in macrophages, particularly regarding M2 activation, during chronic liver diseases such as NASH.

3.7 Increased incidence of HCC in Gaucher disease: A multicentric long-term analysis from 4 German centers

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Introduction Gaucher disease (GD) is a rare monogenic autosomal-recessive disease caused by deficient β-glucocerebrosidase. Intralysosomal accumulation of glucocerebroside leads to ballooning and inflammatory transformation of macrophages. As shown in previous own work (1–3), total cancer incidence among GD patients is increased 2.5-fold and 12.7-fold for hematological tumors as compared to the general population with splenectomized patients being at highest risk (1). The exact incidence of HCC in Gaucher patients and its relationship to pre-existing cirrhosis is unknown.

Methods In a cohort of 249 patients from 4 German centers observed for an average of approx. 12 years (manifestation age 28-68 years), the incidence of HCC and the role of cirrhosis was investigated.

Results In a cohort of 249 patients, 4 new cases of HCC were observed for an average observation time of 11.9 years/patient (mean age at Dx 54 yrs., 2963 pt. years total, m/w=3/1). Out of 4 patients, two had been splenectomized. In 3/4 cases, cirrhosis had not been present prior to advent of HCC. A lethal course within 1-3 years after Dx despite multimodal therapy was observed in 3/4 HCC cases, and one patient developed recurrence of HCC after CT-guided brachytherapy and is currently treated by BSC.

Conclusion Whereas normal incidence of HCC is about 7/100.000 (4), the data from the German Gaucher cohort suggest an increase of HCC incidence to 135/100.000. As incidence of HCC without cirrhosis is significantly increased in Gaucher disease and a deleterious course is frequent, the role of sphingolipids and immune dysregulation related to tumorigenesis in GD remains to be elucidated. Glucosylceramide-laden, chronically activated macrophages are known to release immunologic factors, e.g. interleukins, and accumulation of sphingolipids might contribute to tumorigenesis under these conditions. The results extend studies from previous work (1-3) and as most patients had not been cirrhotic, all GD patients should undergo regular HCC surveillance.

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3.8 Role of 5-lipoxygenase for ammonia-induced astrocyte senescence

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Question Recent studies suggested an important role of 5-lipoxygenase (5-LO) for the induction of senescence in the brain during aging (Manev *et al.* 2000, FASEBJ 14, 1464-9) and showed that astrocytes become senescent in hepatic encephalopathy (HE) (Görg *et al.* 2019, J Hepatol 71, 930-941). Whether 5-LO also contributes to senescence in HE is currently unknown and was investigated in the present study.

Methods Protein levels of total and serine^{505}-phosphorylated cytosolic phospholipase A_2 (cPLA$_2$), 5-LO, Coactosin-like protein 1 (COTL1), NADPH-oxidase 4 (NOX4) serine^{392}-phosphorylated P53, P21 and growth arrest damage inducible protein 45α (GADD45α) were analyzed by immunofluorescence analyses and fluorescence microscopy. Heme oxygenase 1 (HO1), glucose regulated protein 78 (GRP78), P21 and GADD45α mRNA levels were quantified by realtime-PCR. Senescence-associated β-galactosidase (SAβGal) activity was measured using the SAβGal substrate C_{12}FDG and fluorescence microscopy.

Results Cytosolic and nuclear cPLA$_2$ and nuclear P^{Ser505}-cPLA$_2$, 5-LO and COTL1 immunoreactivities strongly increased 24 and 72h after incubating cultured rat astrocytes with NH_4Cl (5mM). Levels of nuclear NOX4 and p^{Ser392}-P53 and HO1, P21 and GADD45α mRNA increased in NH_4Cl (5mM, 72h)-exposed astrocytes. Inhibition of cPLA$_2$ with CAY10650 (1μM) or 5-LO with Zileuton (10μM) inhibited the upregulation of HO1, GRP78, P21 and GADD45α mRNA at 24h but not at 72h after NH_4Cl (5mM)-exposure. The NH_4Cl-induced nuclear accumulation of P21 and GADD45α and elevation of C_{12}FDG fluorescence was completely prevented by Zileuton. Transcriptome analyses data showed that mRNA levels of 5-LO, 5-LO activating protein and COTL1 were upregulated in human *post mortem* brain tissue from patients with liver cirrhosis with HE but not in those without HE.

Conclusions The present study suggests an important role of cPLA$_2$ and 5-LO for ammonia-induced transcription of surrogate markers for oxidative and ER stress and senescence and the nuclear accumulation of P21 and GADD45α and the induction of astrocyte senescence. Elevated mRNA levels of cPLA$_2$, 5-LO, 5-LO activating protein and COTL1 mRNAs in *post mortem* brain samples from patients with liver cirrhosis with HE further suggest that upregulation of 5-LO and 5-LO dependent leukotriene synthesis plays a role for senescence in HE.

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3.9 Gender and microbiota determine hepatic bile acid and metabolic response to a single fast-food meal in healthy adults

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Background and Aim High caloric Western type diet is associated with obesity, the metabolic syndrome and its hepatic manifestation nonalcoholic fatty liver disease (NAFLD). Fast foods are quick alternatives to home cooked meals, high in saturated fat, sugar, salt and calories, which is frequently consumed in excess and represents the preferred food type of several eating disorders including bulimia and binge eating. To date it remains unclear how intermittent excess food intake and especially fast food consumption may influence liver metabolism. Aim of this study was to characterize the effects of a single fast food binge on hepatic steatosis, inflammation, bile acid- (BA), glucose and lipid metabolism.

Methods We recruited 25 healthy individuals without significant alcohol consumption during 14 days prior to inclusion and assessed baseline transaminases, fasting bile acids, lipid profile, glucose and cytokine levels. Additionally, transient elastography as well as controlled attenuation parameter (CAP) to assess hepatic steatosis were performed. Later that day, the subjects received a high caloric fast food meal of their choice and were asked to continue eating for a two-hour period or until fully saturated. The next day, we repeated all the studies mentioned above after a 12h fasting period. Additionally, we collected stool samples for microbiota analysis prior to the food excess.

Results Analysis revealed a modest increase of fasting CAP accompanied by a robust increase of fasting serum BA levels the day after the meal. Surprisingly, serum transaminases, cholesterol and bilirubin levels were significantly lower the day after the meal. When differentiating individuals with a relevant deltaBA>1 vs. individuals without deltaBA≤1, we identified several gut microbiota as well as the individuals’ sex to be associated with BA increase and the observed alterations in liver function, metabolism and inflammation.

Conclusion A single excess fast food meal leads to a robust increase of total serum BA and alterations in several parameters of liver injury and metabolism, indicating a potential acute effect on MDR2 and FXR signaling.

3.10 *MBOAT7* rs641738 POLYMORPHISM IS LINKED TO ALTERED PORTAL BLOOD FLOW ASSESSED USING 13C METHACETIN TEST

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Background *MBOAT7* rs641738 is a common genetic variant associated with non-alcoholic fatty liver disease (NAFLD), inflammation and liver fibrosis, which increases the progression from simple liver steatosis to hepatocellular carcinoma. Liver biopsy is still considered the “gold standard” method in quantifying the degree of liver injury in patients with NAFLD, despite potential complications due to its invasive nature. Consequently, non-invasive tools are seen as alternatives to liver biopsy. Here, we aimed to study the dynamic function of the liver by oral administration of 13C methacetin in individuals carrying the *MBOAT7* rs641738 variant.

Patients and Methods In 92 subjects (age: 43.9 ± 1.5 years, body mass index: 28.8 ± 0.7 kg/m2, females: 42 %, NAFLD: 63 %, obese: 40 %), portal blood flow (delta over baseline value after 15 min: DOB15) and microsomal function (cumulative per cent dose recovery after 30 min: cPDR30) were assessed by oral administration of 13C methacetin. Subjects were stratified according to allele types (wild-type, heterozygous, mutant). Liver steatosis and fibrosis were assessed non-invasively by ultrasonography (degree: 0-3) and acoustic radiation force impulse (ARFI) technique (degree: 0-4), respectively.

Results Mean degree of liver steatosis and liver fibrosis in NAFLD patients was 1.6 ± 0.1 and 1.1 ± 0.2 (mean±SEM), respectively. *MBOAT7* genotypes were neither associated with liver fibrosis nor steatosis. Portal blood flow was significantly lower in homozygous carriers of the *MBOAT7* risk variant as compared to heterozygous subjects (DOB15: 11.1±1.5 vs. 16.4±1.0 ‰, P=0.047), even falling below the cut-off value (DOB15 < 14.5 ‰). Microsomal function tended to be lower in subjects who are homozygous for the *MBOAT7* risk allele for in comparison to heterozygous subjects (9.9±0.9 vs.12.4±0.6 ‰, P=0.065), but remained well above the cut-off value (cPDR30 < 8.1 %).

Conclusions The *MBOAT7* rs641738 variant might be associated with impaired portal blood flow. Microsomal function was preserved but tended to decrease in carriers of the gene variant, too. In the future, more studies should link the *MBOAT7* rs641738 variant with NAFLD severity using non-invasive liver function tests.

3.11 The role of hepatic cholesterol crystals and NLRP3 inflammasome in ABCG5/G8-deficient mice

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The NLRP3 inflammasome plays an important role in metabolic fatty liver diseases (MAFLD). Hepatic damage is attributed, at least in part, to the accumulation of lipid molecules and/or cholesterol crystals. During non-alcoholic steatohepatitis (NASH) *Nlrp3* is upregulated, leading to hepatic inflammation and progression to liver fibrosis, cirrhosis, and hepatocellular carcinoma. The hepatobiliary cholesterol transporter Abcg5/g8 protects the hepatocyte from cholesterol overload and is an important mediator of whole-body cholesterol homeostasis. The aim of the present study was to analyze the sterile inflammatory response by NLRP3/IL-1b in liver- and intestine-specific ABCG5/G8-deficient mice.

Using BAC-recombineering, conditional *Abcg5/g8* knock-out mice were generated allowing tissue-specific deletion of the first two exons of *Abcg5* and the first exon of *Abcg8* by Cre-mediated recombination. *Abcg5/g8* flox mice were crossed to *Villin*-Cre mice, expressing *Cre* in the intestine under the control of the *Villin* promoter (Int-KO), and *Albumin*-Cre mice, allowing hepatocyte-specific deletion of the transporter (Hep-KO). Mice were either fed chow or lithogenic diet (15 % butter fat, 1 % cholesterol, 0.5 % cholic acid), respectively. Expression analyses for *Nlrp3* and *Il1b* as well as histological determination of neutral lipids by Oil Red O staining and cholesterol crystals using polarizing light microscopy were performed.

Under chow diet, *Nlrp3* was decreased in both knock-out mice compared to wild-type (WT) controls, whereas *Nlrp3* was significantly induced when challenged with the lithogenic diet. *Il1b* was not affected in chow-fed mice and showed no difference in HepKO mice upon lithogenic diet feeding but was increased in IntKO mice. Hepatic lipid contents were increased in both knock-out groups fed chow but increased irrespective of genotype on the lithogenic diet. Of note, the amount of cholesterol crystals increased significantly in HepKO upon lithogenic diet challenge, but were not altered in IntKO as compared to WT.

The results show that tissue-specific deletion of *Abcg5/g8* in liver or intestine leads to elevated levels of *Nlrp3, Il1b* and hepatic lipid contents as well as cholesterol crystallization under lithogenic diet. Intrahepatic cholesterol crystals are drivers of hepatic inflammation during NASH by activating the inflammasome. Liver- and intestine-specific ABCG5G5/G8-deficient mice might serve as a new model to study MAFLD/NASH in mice.

3.12 Extracellular vesicles and their associated cargoes as potential biomarker in liver diseases

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Background Extracellular vesicles (EVs) are a heterogeneous group of lipid-enclosed nanoparticles secreted by eukaryotic and prokaryotic cells. EVs are known for participating to intercellular communication and for contributing to diverse cellular processes, such as immune responses and coagulation. EVs have garnered much interest due to their potential utility both as circulating biomarkers in human diseases and as possible delivery agents in targeted therapies. However, EVs small sizes and heterogeneity pose a number of challenges to their isolation, study and characterization.

Methods NTA (Nanoparticle Tracking Analysis), FACS (fluorescence-activated cell sorting) and western blot were used to characterize EVs circulating in the serum of animal models for liver diseases. EVs functionality following isolation was evaluated by in-vitro uptake assay and monitored fluorometrically. To evaluate EVs cargoes as potential source of biomarkers, EVs-associated microRNAs and cytokines were measured. EVs-associated cytokines were quantified in patients with cholangiocarcinoma (CCC) by using Luminex 8plex, whereas EVs-associated microRNAs in patients with non-alcoholic steatohepatitis (NASH) and auto-immune liver diseases (AIH) were measured by miQPCR.

Results Here we show that the amount and size of EVs circulating in the serum of patients with NASH and AIH are significantly different compared to EVs circulating in sera prepared from healthy donors. Furthermore, the expression of several EVs-associated microRNAs was found to be significantly altered. The levels of miR-142-3p, miR-15a and miR-10a were significantly upregulated in EVs circulating in AIH patients. We also discovered that EVs transport a significant amount of cytokines. Specifically, we showed that EVs isolated from CCC patients contain a significantly higher amount of IL2 and IL8 compared to those from the serum of heathy donors.

Conclusions While this is an exploratory, hypothesis-generating study, it shows that the quantitative analysis of EVs can discriminates between healthy and diseased individuals, whereas miR-142-3p, miR-15a and miR-10a might have a potential utility as biomarkers in AIH. Moreover, our data indicate that the analysis of cytokines in EVs might enable for more experimental flexibility. Overall, our goal is, in combination with future studies, to assess the potential of qualitative and quantitative analyses of EVs as predictive and prognostic biomarkers in liver diseases.

3.13 *Lipoprotein and metabolic profiles indicate similar cardiovascular risk of liver steatosis and NASH*

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Question Non-alcoholic fatty liver disease (NAFLD) affects about 25 % of the global population, with no reliable non-invasive tests to diagnose non-alcoholic steatohepatitis (NASH) and to differ between NASH and NAFL (steatosis alone). It is unclear if NAFL and NASH differ in cardiovascular risk for patients. Here, we compared obese NAFLD patients with a healthy cohort, to test, whether cholesterol compounds could represent potential non-invasive markers and to estimate associated risks.

Methods Serum samples of 46 patients with histologically confirmed NAFLD (17 NAFL, 29 NASH) that underwent bariatric surgery were compared to 32 (9 males, 21 female) healthy controls (HC). We analyzed epidemiological data, liver enzymes, cholesterol-, lipid- profile and amino acids. The latter were analyzed by nuclear magnetic resonance (NMR) spectroscopy.

Results Total serum - and HDL- cholesterol were significantly lower in NAFLD groups than in HC with a stronger reduction in NASH. Similar observations were made for sub specification of HDL-p, HDL-s, SHDL-p, and LHDL-p cholesterols. LDL-s and LLDL-p cholesterol were significantly reduced in NAFLD groups. Interestingly, SLDL-p cholesterol was significantly higher in NAFL with a stronger elevation in NASH compared to HC. The amino acids alanine, leucin, and isoleucine were significantly higher in NAFL and NASH compared to HC.

Conclusions We show in this study that cholesterol profiles, apolipoproteins and amino acids could function as a potential non-invasive test to screen for NAFLD or even NASH in larger populations. However, few differences in cholesterol profiles were identified between NAFL and NASH, indicating similar cardiovascular risk profiles.

3.14 Macrophage-specific Fatp4 deletion exacerbates HFHC-induced NASH via a shift towards M2 polarization

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Background Mutations of fatty acid transport protein 4 (FATP4) cause ichthyosis prematurity syndrome (IPS) characterized by skin lesions and associated with eosinophilia and allergies. We hypothesized that FATP4 may have biological functions in immune cells, particularly macrophages, as M2 macrophages are known to mediate allergic inflammation. Therefore, lysM-Cre Fatp4 deficient (KO) mice were generated and subjected to high-fat/high-cholesterol (HFHC) diet as a model of non-alcoholic steatohepatitis (NASH) and chronic stress.

Methods KO and C57BL/6 control mice at one year of age were subjected to HFHC feeding for 16 weeks. Histological analysis of mouse livers was performed. Plasma cytokines and liver transaminases were measured. Lipid assays of plasma and livers were performed.

Results Upon HFHC feeding, male KO mice showed a >2-fold increase of spleen weight. These KO presented a significant increase of liver lipid vesicles, but a decrease of plasma triglycerides (TG) and fatty acids (NEFA). Under HFHC feeding, female KO mice showed a moderate increase of liver and spleen weights. By histological analysis of livers, these female mice displayed massive infiltration of immune cells. These mice showed a decrease of TG and NEFA in livers and plasma. Thus, macrophage-specific Fatp4 deficiency under HFHC induced more prominent splenomegaly in males. While male mutants presented increased liver steatosis, female mutants showed decreased liver lipids associated with accumulation of immune cells. HFHC-fed KO mice showed an increase of plasma M2/Th2 markers IL-4 and IL-13 by ~5 folds for females and by ~3 and ~4 folds for males, respectively. Both male and female mutants showed an increase of chemotactic factor MCP-1, whereas only female mutants presented an elevation of IL-5 and IL-6. Hence, compared with male mutants, female mutants showed a larger increase of IL-4 and IL-13 as well as an additional increase of IL-5 and IL-6. Notably, TNF-α was significantly decreased in plasma of both male and female mutants, supporting a shift from M1 towards M2 polarization upon HFHC feeding.

Conclusions Under HFHC, macrophage Fatp4 deficiency in males exacerbated hepatic steatosis, whereas the deficiency in females induced progression to NASH. While the increase of M2/Th2 markers is consistent with allergies, the increase of eosinophil chemoattractant IL-5 observed in female mutants is in line with eosinophilia reported in IPS patients with FATP4 mutations.

3.15 Ammonia triggers astrocyte senescence through glucosamine synthesis-dependent upregulation of ASCT2 and KGA

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Question Recent studies point to an important role of glucosamine synthesis-dependent astrocyte senescence for persistent cognitive impairment in patients with liver cirrhosis and hepatic encephalopathy (HE) (Görg *et al.* GLIA 2015, 107: 1475-1480 and J Hepatol 2019, 71: 930-941). In the present study we investigated a potential role of the glutamine transporter ASCT2 and the kidney-type glutaminase (KGA) for ammonia-induced astrocyte senescence.

Methods KGA and ASCT2 mRNA and protein levels were analyzed by qPCR, Western blot and immunofluorescence analysis, respectively. ASCT2, KGA, GFAT1/2 proteins were downregulated in rat astrocytes *in vitro* using specific siRNAs. The activity of senescence-associated β-galactosidase (SAβGal) was measured using the SAβGal substrate C_{12}FDG and fluorescence microscopy.

Results Immunofluorescence analysis, confocal laserscanning and superresolution microscopy showed that KGA and glutamine synthetase (GS) are present in one and the same astrocyte in culture and in rat brain and that ASCT2 and KGA are both found in mitochondria. NH_4Cl (5mM, 72h) upregulated KGA protein and ASCT2 protein and mRNA levels in cultured astrocytes. Upregulation of KGA protein by NH_4Cl was completely prevented by the GS inhibitors methionine-sulfoximine (3mM) and phosphinothricin (100μM). siRNA-mediated knockdown of GFAT1/2 inhibited the NH_4Cl-induced upregulation of KGA protein and ASCT2 mRNA and protein. Knockdown of KGA or ASCT2 by specific siRNAs inhibited the upregulation of surrogate markers for oxidative and endoplasmic reticulum stress (heme oxygenase 1, glucose regulated protein 78) and senescence (growth arrest and DNA damage inducible 45α) in NH_4Cl-exposed astrocytes. Knockdown of KGA also prevented the enhanced SAβGal activity and astrocyte proliferation inhibition in NH_4Cl-exposed astrocytes. KGA protein levels were also elevated in rat cerebral cortex after inducing hyperammonemia through intraperitoneal injection of ammonia acetate.

Conclusion Our findings suggest that ammonia triggers astrocyte senescence through glucosamine synthesis-dependent upregulation of ASCT2 and KGA and ASCT2-dependent glutamine import into mitochondria and subsequent KGA-dependent glutaminolysis. These findings may have important implications for senescence-induced persistent cognitive impairment in patients with liver cirrhosis and HE.

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3.16 Identification of ammonia-induced changes in the astrocyte *O*-GlcNAcome

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Question Glutamine accumulation in astrocytes is a hallmark of the pathogenesis of hepatic encephalopathy (HE) (Häussinger *et al.* 1994, Gastroenterology 107, 1475-1480) and recent studies suggest a central role of the glutamine-dependent *O*-GlcNAcylation of so far unknown proteins for astrocyte

dysfunction in HE (Görg *et al.* 2019, J Hepatol 71, 930-941). The aim of the present study was to identify comprehensive changes in the *O*-GlcNAcome in ammonia-exposed astrocytes *in vitro* and to identify potential consequences of the *O*-GlcNAcylation for the functions of the respective proteins.

Methods *O*-GlcNAcomics were performed by mass spectrometry on Click-iTTM chemistry-purified *O*-GlcNAcylated proteins. Individual *O*-GlcNAcylated proteins were identified by Click-iTTM and Western blot and heme oxygenase 1 (HO1) interacting proteins were detected by chemical crosslinking with 3,3-dithio-bis-(sulfosuccinimidyl)propionate and Western blot analysis. Purified recombinant HO1 was *O*-GlcNAcylated *in vitro* using recombinant *O*-GlcNAc-Transferase (OGT).

Results Using Click-iT^{TM} chemistry and mass spectrometry we identified 871 proteins of the astrocytic *O*-GlcNAcome. The *O*-GlcNAcylation of 51 and 17 of these proteins was enhanced or reduced in NH_4Cl (5mM, 72h)-exposed cultured rat astrocytes, respectively. By Click-iT^{TM} and Western blot analysis we further identified HO1 to be *O*-GlcNAcylated in NH_4Cl-exposed astrocytes. After crosslinking interacting proteins in cultured astrocytes, we detected a large number of anti-HO1 immunoreactive proteins by Western blot in NH4Cl-exposed astrocytes with molecular weights between 25-250kDa. Some of these matched with molecular weights of HO1 oligomers and HO1 oligomers also formed upon *in vitro* *O*-GlcNAcylation of purified rat HO1 using recombinant OGT. Bioinformatic analyses predicted serine^{160} as a potential *O*-GlcNAcylation site in HO1 and showed that it is located at the center of a putative HO1 dimerization site.

Conclusions In the present study we identified a variety of proteins whose *O*-GlcNAcylation is altered by ammonia in cultured astrocytes. *O*-GlcNAcylation of HO1 triggers HO1 oligomerization which is suggested to enhance HO1 catalytic activity. As HO1 is central for the induction of astrocyte senescence in ammonia-exposed astrocytes, HO1 *O*-GlcNAcylation may be of relevance for the pathogenesis of HE.

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3.17 Gender differences in Western-type diet-induced alterations of lipid metabolisms in the liver and adipose tissue of mice

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It is increasingly recognized that beyond alterations in hepatic lipid metabolisms, the pathogenesis of non-alcoholic fatty liver disease (NAFLD) is closely related to insulin resistance and dysfunctionality of adipose tissue.

The aim of this study was to assess gender differences within these complex disturbances of components of the metabolic syndrome in an experimental mouse model.

Methods and results A Western-type diet (WTD; 38 % fat, 30 % sucrose and 0.2 % cholesterol) supplemented with 30 % fructose in drinking water [CH1] was applied to 8 week-old male and female mice for 20 weeks. Littermate controls were fed with standard chow. WTD-induced weight gain and visceral adipose tissue (VAT) mass was significantly higher in male than in female mice, and only WTD-fed male mice showed pathological glucose tolerance. Furthermore, expression of pro-inflammatory factors IL-6 and MCP-1 was significantly higher in VAT of male mice. In contrast, liver/body weight ratio und hepatic triglyceride content did not significantly differ between sexes, although female mice showed significantly higher hepatic expression of the fatty acid transporter CD36, fatty acid synthase and nicotinamide phosphoribosyl transferase (NAMPT), an enzyme involved in the development of hepatic steatosis. Moreover, female mice had significantly higher hepatic HMG-CoA reductase (HMGCR) and ATP-binding cassette transporter subfamily A member 1 (ABCA1) expression levels, and fitting to this, significantly higher hepatic cholesterol as well as primary and secondary bile acid levels. Furthermore, induction of oxidative stress and pro-inflammatory gene expression were higher in WTD-fed female mice.

Conclusion Our study reveals significant gender-specific differences in fatty acid accumulation and pro-inflammatory gene expression in VAT on the one side and hepatic lipid, cholesterol and bile-acid metabolisms on the other side. Future studies are needed to verify these mechanisms in men. Potentially, this could lead to the identification of new sex-specific therapeutic targets to prevent NAFLD development and its progression.

3.18 The sexual dimorphism of primary murine hepatocytes changes during cultivation

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Question The liver is one of the most sexual dimorphic organs. Cholesterol, fatty acid and drug metabolism are among the signal pathways in the liver that are most affected by sexual dimorphism. This in turn influences the development and course of various liver diseases e.g. non-alcoholic fatty liver disease (NAFLD) and hepatocellular carcinoma (HCC), as well as the general drug treatment in male and female organisms. It is therefore highly important to understand the underlying mechanisms of sexual dimorphism and to take this into account when researching diseases and their treatment. For this reason, we investigated the development of sexual dimorphism in primary hepatocytes during a 96 hour cultivation.

Methods Primary hepatocytes from male and female C57Bl6/N mice were isolated and cultured in collagen coated 6-well plates. Samples were taken at 0, 24, 48, 72 and 96 h. We performed transcriptome, proteome, extracellular metabolome and qPCR analyses to examine the samples.

Results We observed that the gene expression of fatty acid beta oxidation was higher from 0 to 24 h in male compared to female hepatocytes, while it was higher in female hepatocytes from 48 to 96 h. In addition, our results show that the sex specific gene expression of several important cytochrome P450 family members was lost during cultivation e.g. *Cyp1a2, Cyp2b23* and *Cyp2b9*. Furthermore, our data revealed that female hepatocytes consumed more amino acids than male ones. Considering all measured signaling pathways, the sexually dimorphic gene expression pattern of 96 h was most consistent with the initial situation at 0 h.

Conclusions Our results show, how the sex-specific regulation of various central metabolic pathways proceeded during cultivation. Furthermore, the data show that there are large differences in the alteration of sex-specific regulation of drug metabolism during the cultivation of primary hepatocytes, which can have an immense influence on the assessment of pharmacodynamic processes. These results should be taken into account when designing new sex-specific pharmacological liver treatments, which are actually of high interest in the term of personalized medicine.

3.19 Prolyl-hydroxylase inhibition ameliorates NAFLD-like hepatocyte alterations via OATP1B1

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Background Nonalcoholic fatty liver disease (NAFLD), comprising a sequence of steatosis, non-alcoholic steatohepatitis (NASH), and liver fibrosis, significantly increases surgical morbidity in patients undergoing liver resection. Improving liver function in NAFLD patients is therefore of particular importance. We previously demonstrated that the prolyl hydroxylase (PHD) small-molecule inhibitor ethyl-3,4-dihydroxybenzoate (EDHB) promotes liver regeneration, putatively via organic anion transporting polypeptide 1B1 (OATP1B1). Here, we investigate whether this effect can be exploited in the setting of NAFLD.

Methods Huh7 cells were stimulated with palmitic acid (PA) for 48 hours to induce NAFLD-like alterations. EDHB-treatment of cells was performed upon the siRNA-mediated knockdown of OATP1B1 or control transfection. The effects of EDHB treatment were assessed by quantification of changes in lipid metabolism, apoptosis, and fat accumulation.

Results PA treatment expectedly caused lipid accumulation, apoptosis, and lipid metabolism in Huh7 cells. Pretreatment with EDHB significantly mitigated lipid aggregation, improved fatty acid oxidation, and reduced LDH release and cell apoptosis. These protective effects of EDHB could be abrogated by the simultaneous knockdown of OATP1B1.

Conclusions EDHB ameliorates NAFLD-like hepatocyte alterations in an *in vitro* model. This effect is likely dependent on hepatocyte expression of OATP1B1.

3.20 NAFLD/NASH predict overall but not cardiovascular mortality in patients with medium-to-high cardiovascular risk

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Untreated NAFLD may have significant consequences including an increase in mortality and cardiovascular injury. Thus, early detection of NAFLD is currently believed not only to prevent liver related but also cardiovascular mortality. However, almost nothing is known about the relevance of co-existing NAFLD in patients with angiographically validated coronary artery disease (CAD).

We investigated the impact of NAFLD in a large cohort of patients that had been referred to coronary angiography. Modelling the common NALFD and fibrosis scores FIB-4 and NFS as splines revealed significant associations with all-cause and cardiovascular mortality when the Cox regression models were only adjusted for cardiovascular risk factors that were not already included in the calculation of the scores. Stratifying the scores into quartiles yielded hazard ratios (95 % CI) for all-cause and cardiovascular mortality for the 4th quartile vs the 1st quartile of 2.28 (1.90-2.75) and 2.11 (1.67-2.67) for FIB-4 and of 3.21 (2.61-3.94) and 3.12 (2.41-4.04) for NFS.

However, we did not observe an independent association of FIB-4 or NFS with cardiovascular mortality in our prospective CAD cohort after full adjustment for all cardiovascular risk factors. Regarding all-cause mortality, the spline model remained significantly associated while the model using quartiles was not.

To further validate these observations, we restricted this analysis to a cardiovascular (CV) high-risk group of 2215 patients with more than 20 % luminal narrowing of a coronary artery as confirmed by coronary angiography. Again, even in these CV high-risk profile patients, NAFLD relevant scores did not predict cardiovascular mortality. Also, neither NFS nor FIB-4 were useful as prognostic models for primary prevention of cardiovascular events.

In summary, our results from a well-defined, prospective CAD as well as from a defined high CV risk subgroup demonstrated that concomitant NAFLD was not an independent predictor of cardiovascular mortality.

Lectures Session IV Tumors
Saturday, January 30, 2021, 9:15 am – 10:00 am, Lecture Hall Virtual Venue

4.1 Cyclin E1 and Cdk2 in Hepatic Stellate Cells are critical for initiation and progression of liver fibrosis and cancer

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Question Hepatocellular carcinoma (HCC) is one of the most severe tumor diseases with increasing incidence and limited treatment options. Typically, HCC develops during a multistep process involving chronic liver inflammation and liver fibrosis. The latter is characterized by accumulation of extracellular collagen produced by Hepatic Stellate Cells (HSCs). This process involves cell cycle re-entry and proliferation of the normally quiescent HSCs. The cell cycle is regulated by cyclins and associated cyclin-dependent kinases (Cdks). In the present study, we examined the role of Cyclin E1 (CcnE1) and Cdk2 in HSCs for liver fibrogenesis and hepatocarcinogenesis.

Methods We crossed conditional CcnE1 ($CcnE1^{f/f}$) or Cdk2 ($Cdk2^{f/f}$) knockout mice with transgenic mice expressing cre-recombinase under the control of the L-rat promoter resulting in mice lacking CcnE1 or Cdk2 specifically in HSCs ($CcnE1^{\Delta HSC}$ or $Cdk2^{\Delta HSC}$). $Cdk2^{\Delta HSC}$ and $CcnE1^{\Delta HSC}$ mice were challenged with CCl_4 for 6 weeks and subsequently investigated for liver fibrosis. Induction of fibrosis and HCC in $CcnE1^{\Delta HSC}$ mice was performed using the DEN/CCl_4 model.

Results Genetic ablation of Cdk2 or Cyclin E1 specifically in HSCs significantly reduced collagen accumulation and fiber formation in the liver after CCl_4 treatment when compared to cre-negative littermates. Accordingly, $Cdk2^{\Delta HSC}$ and $CcnE1^{\Delta HSC}$ mice showed a significantly reduced HSC activation in the liver, as determined by aSMA and col1a1 gene expression. This suggests that cell cycle re-activation of naïve HSCs *in vivo* requires functional Cdk2 presumably in complex with Cyclin E1. $CcnE1^{\Delta HSC}$ mice, challenged with DEN/CCL_4 showed a reduced number and size of dysplastic lesions when compared to cre- littermates, which was again associated with decreased HSC activation even after long-term observation.

Conclusion We provide evidence that the pro-fibrotic properties of HSCs depend on functional Cdk2 and CcnE1. In addition, inhibition of Cyclin E1 in HSCs is sufficient to reduce initiation and progression of HCC in mice at least in the DEN/CCL_4 model. We conclude that a HSC-specific therapeutic targeting of Cyclin E1 or Cdk2 in patients with liver fibrosis and high risk for HCC development could be beneficial. In summary, our study contributes to a better understanding of the complex, cell type-specific roles of CcnE1 and Cdk2 for the pathological sequence from liver fibrosis to HCC.

4.2 T cell exhaustion dynamics are linked to clinical outcomes in Hepatocellular Carcinoma

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Question Hepatocellular Carcinoma (HCC) is a rising global health problem with limited treatment options and poor prognosis. Despite translation of immunotherapy into clinical parameters the immunobiology of hepatocellular carcinoma is poorly understood. Exhausted and liver-resident T cells have been identified in HCC patients but their role and clinical relevance remains unclear. We therefore aimed to assess the clinical impact of T cell exhaustion in HCC patients by using a system immune profiling approach based on newly developed transcriptomic and cytometric signatures.

Methods Comprehensive phenotypic and functional exhaustion profiling of the peripheral and intrahepatic immune compartment (tumor-surrounding and tumor microenvironment) was performed ex vivo on samples from 20 HCC patients using mass cytometry. Single-cell- and population based transcriptome profiles from published datasets of HCC resections were in silico assessed for signatures of T cell exhaustion and residency and their link to patient survival. Exhaustion and residency of exhausted T cells were further analyzed by immunohistochemistry and imaging mass cytometry.

Results High parametric phenotypic and functional analysis identified a significant enrichment of exhausted CD8+ T cells (TEX) in the tumor microenvironment. Interestingly, a subgroup of mainly PD-1 positive TEX showed a strong co-expression of CD103, a marker for tissue resident memory cells (TRM). Analysis of the relationship between TEX and TRM revealed a high enrichment of TEX at the expense of TRM linked to poorer patient survival. Of note these TEX showed a differential expression of multiple immune checkpoint targets in the tumor microenvironment. In line with a potential protective role of TRM, survival analysis indicated that a higher TRM fraction of TEX was positively associated with progression free survival. These findings were confirmed by in silico analysis of exhaustion and residency signatures in TCGA HCC samples.

Conclusion These data demonstrate the clinical relevance of an exhausted T cell response in HCC patients. The dynamics between exhausted and resident liver T cells have implications for immune-based diagnostics, rational patient selection and monitoring during HCC immunotherapies. Based on these findings we propose the displacement of TRM by TEX in HCC as a novel clinically relevant pathophysiological concept.

4.3 KRAS-mutated iCCA display distinct molecular alterations and a preferential sensitivity towards PARP-1 inhibition

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DOI 10.1055/s-0040-1722040

Introduction Intrahepatic cholangiocarcinoma (iCCA) is the second most common primary liver cancer with an increasing incidence over recent years. Due to the complexity of iCCA pathogenesis and the pronounced genetic heterogeneity treatment options are still limited. KRAS mutations are observed in a sizeable subgroup of iCCA patients and characterized by poor response to chemotherapy and reduced overall survival, highlighting the need for novel therapeutic approaches. Several findings indicate that DNA damage response protein Poly (ADP-ribose) polymerase 1 (PARP-1) preferentially affects KRAS-mutated iCCA cell lines, but the exact mechanism of PARP-1 in cholangiocarcinogenesis remains elusive.

Material & Methods siRNA-mediated knockdown of PARP-1 and treatment with PARP-1-inhibitor AZD2281 were conducted in KRAS-mutated and non-mutated iCCA cell lines. Functional assessment of PARP-1 knockout and inhibition on tumorigenic potential was analyzed by viability assay and colony and sphere formation. Further, we examined cellular reactive oxygen species (ROS) levels using DCFDA assay. Further, we used a PARP-1 knockout mouse model inducing specific KRAS-mutated tumors by hydrodynamic tail vein injection (HDTV). Transcriptome analyses of CRISPR/Cas9 PARP-1 knockout clones were employed to further investigate underlying molecular mechanisms.

Results siRNA-mediated knockdown of PARP-1, as well as treatment with PARP-1 inhibitor preferentially impaired cell viability and tumorigenicity in KRAS-mutated cell lines and led to a 40-45 % reduction in colony and sphere formation capacity. Further, KRAS-mutated cell lines showed higher basal ROS levels after PARP-1 knockout and higher generation of ROS after induction of oxidative stress via H2O2 treatment. *In vivo* induced tumors induced by injection of KRAS/p53 showed a distinct phenotype in PARP-1 deficient background. Transcriptome analyses of CRISPR/Cas9 PARP-1 knockout clones revealed differential expression of DNA damage response pathways as well as other cellular pathways known to be affected by PARP-1, e.g. oxidative stress, c-MYC, and cell death signaling.

Conclusion This study confirms a preferential sensitivity of KRAS-mutated iCCA towards PARP-1-based interventions. PARP-1 inhibition induces functional impairment of tumorigenic capacity and induction of oxidative stress. These findings suggest an unrecognized therapeutic role of PARP-1 in this poor prognostic subgroup of iCCA patients.

Lectures Session V Viral Hepatitis and Immunology
Saturday, January 30, 2021, 11:45 pm – 12:30 pm, Lecture Hall Virtual Venue

5.1 Molecular mimicry is not a sufficient cause of autoimmune liver disease in mice

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DOI 10.1055/s-0040-1722041

Question A prevalent concept to explain the occurrence of autoimmunity is cross-recognition of microbial and self-antigens, i.e. molecular mimicry. However, the actual role of molecular mimicry in disease development remains

controversial. While it is suggested to activate autoreactive T cells, it might also induce a suppressive response, such as by Tregs and thus protect from autoimmune disease.

Methods We have previously generated a mouse model in which an MHC class II-restricted CD4 T cell epitope of LCMV (GP61-80) is expressed in hepatocytes (Alb-iGP). Alb-iGP mice do not develop autoimmune liver disease (ALD); however, when crossed to Smarta mice featuring T cells recognizing GP61-80, the resulting Alb-iGP_Smarta mice spontaneously develop ALD after 20 weeks of age. To study the harmful or protective effects of molecular mimicry, we infected Alb-iGP and Alb-iGP_Smarta mice at 8 weeks of age with 10^6 FFU LCMV-WE, monitored the incidence of ALD and analyzed the GP61-80-specific T cell response.

Results Following viral clearance, Alb-iGP mice maintained an enlarged population of antigen-specific CD4 T cells in liver (0.75 % vs. 0.09 %) and spleen (0.60 % vs. 0.05 %) up to the age of 52 weeks as compared to non-infected controls. Despite the presence of potentially autoreactive CD4 T cells, these mice did not develop ALD. ALD resistance was associated with Treg expansion that amounted to 25 % of antigen-specific T cells in the liver as compared to 5 % Tregs in non-specific CD4 T cells. In contrast, ALD-prone Alb-iGP_Smarta mice remained chronically infected, presumably due to reduced CD8 T cell numbers, but were protected from ALD. Similar to Alb-iGP mice, ALD resistance of infected Alb-iGP_Smarta mice was associated with a 5-fold increase in antigen-specific Tregs and reduction of antigen-specific CD4 effector T cells as compared to non-infected controls; moreover, the remaining effector cells displayed an exhausted phenotype.

Conclusion Acute infection of Alb-iGP mice induced lasting expansion of potentially self-reactive CD4 T cells. However, these mice were protected from ALD, presumably due to a high proportion of antigen-specific Tregs. Chronic viral infection of Alb-iGP_Smarta mice was accompanied with T cell exhaustion, a loss of antigen-specific effector T cells and an increase in antigen-specific Tregs, which prevented development of ALD. Thus, molecular mimicry provoked a suppressive response and was thus not a sufficient cause of ALD in mice.

5.2 Shutdown of HBV transcripts using siRNA followed by entry inhibition induces sustained silencing of the cccDNA in vivo

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Question There is an urgent need for therapeutic strategies repressing the hepatitis B virus (HBV) nuclear template, the covalently closed circular DNA (cccDNA), since silencing of the viral reservoir may promote functional cure of chronic HBV infection. The structural maintenance of chromosomes 5/6 complex (SMC5/6) is a host factor with the potential to suppress cccDNA activity. However, SMC5/6 is antagonized by the viral regulatory HBx protein, which triggers its degradation. We aimed to investigate the impact of an siRNA that targets all HBV transcripts for degradation on HBx and SMC5/6. In particular, we assessed whether siRNA and other interventions able to lower HBV transcripts can achieve silencing of cccDNA transcription in vivo.

Methods HBV-infected human liver chimeric mice were treated with siRNA for 4 weeks. Virological and host changes were analyzed at the end of treatment and during follow-up by qRT-PCR, ELISA, immunoblotting and chromatin immunoprecipitation (ChIP). RNA in situ hybridization was combined with immunofluorescence to detect SMC6 and HBV RNAs at the single cell level. The entry inhibitor Myrcludex-B was used during the follow-up phase to avoid new infection events. In addition, some mice received pegylated interferon alpha (peg-IFNα) or beta (peg-IFNβ) treatment, which have been shown to affect cccDNA activity.

Results siRNA strongly reduced all HBV transcripts and viral markers in the serum and liver except cccDNA. Interestingly, HBx protein levels were also reduced, thus enabling the reappearance of SMC5/6 in the liver of treated mice. RNA-ISH+IF analysis revealed that the reappearance of the SMC5/6 complex was restricted to hepatocytes negative for HBV transcripts and SMC5/6 was found associated with the cccDNA in ChIP assays. Also peg-IFNα and beta peg-IFNb reduced HBx proteins, promoted SMC5/6 reappearance and achieved cccDNA silencing. However, the antiviral effects did not persist off treatment and SMC5/6 was degraded again regardless of the treatment used. Remarkably, blockade of viral entry during follow-up hindered renewed degradation of SMC5/6 and maintained cccDNA transcriptional silencing.

Conclusions Our results reveal that interventions abrogating HBx promote SMC5/6-mediated silencing of the cccDNA minichromosome, while strategies shielding the human hepatocytes from reinfection are needed to maintain cccDNA silencing.

5.3 Extracellular vesicles of patients with liver cirrhosis & acute-on-chronic liver failure have immunomodulatory functions

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Background and Aims Patients with liver cirrhosis (LC) and acute-on-chronic liver failure (ACLF) show distinct changes regarding the cellular structure of the liver and the immune system. Cell-cell communication and interactions of different liver cell subtypes and immune cells play an important role during disease progression and complications (e.g. infections). Within the last years, the role of extracellular vesicles (EVs) as communicators between cells is raising more and more attention. We therefore aimed to characterize phenotype and function of EVs derived from the blood of LC and ACLF patients.

Method Healthy donor blood from discarded buffy coat bags was provided by the blood donation center at the University hospital Essen. Patients with compensated or decompensated LC and ACLF were recruited from a prospective cohort study. EVs were isolated from EDTA blood by precipitation and characterized regarding particle size and concentration by Nanoparticle Tracking Analysis (NTA). Surface marker were investigated using flow cytometry and protein content was analyzed using Western Blot and Mass Spectrometry. For functional analysis, 20 µg blood-derived EVs were used to stimulate either healthy donor peripheral blood mononuclear cells (PBMCs) or magnetic bead-isolated monocytes for 24h, which were then analyzed by flow cytometry or qPCR.

Results EVs of LC and ACLF patients show lower expressions of exosome-specific marker (e.g. CD9, CD63, CD81) and liver cell-specific marker (e.g. ASGPR1, CD163) compared to healthy donor EVs. Particle concentration in blood of patients decreases, whereas the average size increases with the severity of the disease. In functional assays with PBMCs, blood-derived EVs of LC and – to a stronger extend – of ACLF patients lead to changes in the composition of immune cell populations, e.g. by downregulation of CD197 (lymph node-homing receptor) on T cells or increased expression of activation markers on B cells (CD38, HLA-DR).

Conclusion LC and ACLF patients have less differentiated EVs than healthy donors. The vesicles show immunomodulatory functions for example by leading to an increased amount of $CD4^+$ and $CD8^+$ effector memory T cells.

Poster Visit Session IV Tumors
Saturday, January 30, 2021, 8:30 am – 9:15 am, Poster Session Virtual Venue

4.4 Role of the bile acid receptor TGR5 (GPBAR1) in cholangiocarcinoma (CCA) progression and tumor spread

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DOI 10.1055/s-0040-1722044

Background The membrane bound G-protein coupled bile acid receptor TGR5 is expressed in liver epithelial cells. Its activation through bile acids (BAs) triggers secretion, proliferation and anti-apoptotic effects in normal cholangiocytes and CCA cell lines. TGR5 was found to be overexpressed in human cholangiocarcinoma tissue (CCA) and cell lines generated from CCAs. Especially secondary bile acids play a role in the development of different malignant tumors in the gastrointestinal tract, including liver. Whether TGR5 activation also promotes invasiveness and metastasis development of CCA is unclear and aim of this work. Furthermore, TGR5 and the bile acid receptor S1PR2 show partly overlapping signalling functions in biliary epithelial cells. To date, it is unclear whether there is a potential crosstalk of both receptors.

Methods In the human CCA cell line TFK-1, the CRISPR/Cas9 mediated TGR5 knockout was achieved via nucleofection using sgRNAs that specifically mutate the transmembrane domain 3 (TMD3). Puromycin selected clonal cells were analyzed by Sanger sequencing, quantitative Realtime PCR, immunofluorescence staining and additionally by homology modelling. Furthermore, receptor stimulations of Cas9 mediated TGR5 knockout and control cells with BAs and a TGR5 agonist were performed to measure the proliferative response using BrdU-incorporation assay, as well as migratory and invasive properties using transwell cell migration and invasion assays.

Results/Conclusion Using CRISPR/Cas9 technique, we generated a TGR5 deletion variant Δ89-110, lacking 57 bp within TMD3. While protein expression and plasma membrane localization was unaffected, bile acid and agonist induced cell proliferation, migration and invasion was abolished in TGR5Δ89-110 expressing cells. Modelling of the truncated receptor revealed complete obstruction of the receptor binding pocket by the shortened TMD3, rendering receptor activation impossible. Together, these data indicate that TMD3 is essential for BA mediated TGR5 activation. Moreover, TGR5 truncated CCA cells were resistant to BA induced cell proliferation, migration and invasion, underscoring the role for TGR5 in CCA progression and tumor spread. S1PR2 can induce proliferation and invasion in CCA cells as well. Elevated S1PR2 expression levels in TGR5 truncated cells imply a compensational crosstalk of both bile acid receptors TGR5 and S1PR2, nevertheless S1PR2 elevation cannot fully restore the TGR5 effects.

4.5 ACSL4 and its tumor protective role in chronic liver disease

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DOI 10.1055/s-0040-1722045

Question The prevalence of non-alcoholic fatty liver disease, ranging from steatosis to non-alcoholic steatohepatitis (NASH), is increasing in developed countries. In some patients, progression towards cirrhosis and hepatocellular carcinoma (HCC) occurs. At present, the underlying mechanisms for disease progression remain incompletely understood. In NASH oxidative stress and lipid peroxidation constitute prominent features and may hence play a key role. Interestingly, accumulation of lipid peroxides can trigger ferroptosis, an iron-dependent mode of cell death. According to *in vitro* studies, acyl-CoA synthetase long-chain family member 4 (ACSL4) is an essential contributor to ferroptosis. In our study, we aimed to investigate the relevance of ferroptosis for disease progression using hepatocyte-specific ACSL4 inhibition in experimental models of chronic liver disease.

Methods Primary hepatocytes from either wild-type (WT) mice or animals with hepatocyte-specific deletion of ACSL4 (ACSL4Δhepa) were treated with specific inducers (e.g., RSL3) and inhibitors (e.g., Liproxstatin-1) of ferroptosis. In addition, we compared disease development between WT and ACSL4Δhepa mice to investigate the role of ferroptosis. We used STZ (Streptozocin) with high-fat diet as a NASH-HCC model.

Results Treatment of primary hepatocytes with RSL3 leads to increased cell death, which could be rescued by adding Liproxstatin-1 or by using ACSL4-deficient hepatocytes. In our NASH-HCC model, inhibition of ferroptosis in hepatocytes reduces the severity of chronic liver disease as evidenced by decreased serum transaminase levels in ACSL4Δhepa mice. Importantly, while the overall tumor burden was not affected in ACSL4Δhepa mice, the number of smaller tumors was significantly increased. Interestingly, infiltration of immune cells, especially macrophages and granulocytes, was significantly decreased in ACSL4Δhepa mice. Moreover, earlier evaluation of tumorigenesis showed more tumors as well as higher tumor burden in ACSL4Δhepa mice.

Conclusion Our results demonstrate that primary mouse hepatocytes are susceptible to induction of ferroptosis and that this mode of cell death depends on functional ACSL4. Interestingly, ferroptosis has a protective mechanism during tumor initiation, by regulating lipid accumulation and cell death in hepatocytes. Therefore, activation of ferroptosis or inhibiting key molecules regulating cell death, can be a possible therapeutic treatment for human diseases.

4.6 Interleukin-17 enhances the risk for tumor development in chronic sclerosing cholangitis

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DOI 10.1055/s-0040-1722046

Background Primary sclerosing cholangitis (PSC) is a chronic inflammatory liver disease, which leads to cirrhosis and increases the risk of hepatobiliary malignancy, e.g. hepatocellular carcinoma (HCC). PSC pathogenesis remains largely unknown, but patients bear increased numbers of IL-17A-producing CD4+ T cells. In this study we investigated the *in vivo* effect of IL-17 deficiency on inflammation, fibrosis and tumor development in a mouse model of sclerosing cholangitis.

Methods We analyzed B6-Mdr2-/- mice, an established mouse model of sclerosing cholangitis, crossed with IL-17 deficient animals at various time points (5, 36 and 66 weeks). Severity of liver fibrosis was determined by serum transaminases and quantification of Sirius red stained liver sections. Gene expression was analyzed by qPCR and flow cytometric analysis of hepatic and splenic lymphocytes was performed. Liver inflammation was quantified using the modified hepatic activity index (mHAI) score. Onset and growth of hepatic tumors was analyzed via small animal MRI scans. Western Blot analysis was performed to quantify Stat3 activation in liver tissue.

Results Deficiency in IL-17 significantly reduced liver fibrosis in mice analyzed at 5 weeks of age. In addition, collagen 1A1 levels were significantly decreased and flow cytometric analysis revealed reduced numbers of neutrophils within liver tissue of 5-week-old Mdr2-/- IL17-/-mice, compared to Mdr2-/- mice. Importantly, the observed difference vanished with age resulting in similar

fibrosis levels in Mdr2-/- and Mdr2-/- IL-17-/- mice at the age of 36 and 66 weeks. Moreover, mHAI score, expression of collagen 1A1 and serum transaminases were not altered between the two analyzed groups at these time points. However, although the lack of IL-17 did not seem to affect fibrosis with age, we observed an increased HCC progression at the age of 66 weeks in Mdr2-/- IL-17 competent animals. HCC progression was associated with increased pStat3 levels, enhanced proliferation of hepatocytes and increased IL-6 levels in IL-17 competent Mdr2-/- animals.

Conclusion Several studies suggest tumor-promoting effects of IL-17 in the development, progression and recurrence of HCC in patients. We here demonstrate that IL-17 itself does not affect fibrosis development but significantly enhances the risk of developing HCC by activation of known pro-tumorigenic pathways. This work should stimulate further research on IL-17 in the setting of chronic inflammation.

4.7 LINC00152 regulates KLC2 via sponging of miR143/KLC2 thereby promoting proliferation of human liver cancer cells

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Long non-coding RNAs (lncRNAs) play pivotal roles in multiple cellular processes by regulating gene transcription and protein turnover. In particular, lncRNAs can work as sponge for miRNAs, thereby indirectly regulating miRNA target gene expression. We have previously identified a methylation-dependent upregulation of LINC00152 in human hepatocellular carcinoma (HCC) by integration of methylome and gene expression profiling data. Here, we explored the competing endogenous RNA (ceRNA) network driven by LINC00152 in human HCC.

An *in silico* approach predicted 22 miRNAs potentially binding to the LINC000152 RNA sequence. In the next step, 2664 genes were predicted by at least two algorithms to be targets of the identified miRNAs. The association between the expression of the candidate miRNAs and their putative mRNA candidates was analyzed in a human HCC cohort (n=40) to derive the components of the LINC00152-driven ceRNA network. As an experimental validation, the presence of the top 10 ranked miRNAs was confirmed in LINC00152 ribonucleoprotein complexes (miRNPs) by RNA immunoprecipitation of HuH7 and HepG2 cells. Furthermore, quantitative RT-PCR revealed that the expression level of predicted genes was significantly downregulated in LINC00152 knockout HuH7 cell clones. One of the most significantly altered genes was Kinesin Light Chain 2 (KLC2), which was upregulated in HLE cells overexpressing LINC00152. Analyses of the 3´UTR of KLC2 showed a potential binding site for miR143-3p, which was also found enriched in the miRNPs with LINC00152. As expected, transfection of the specific miRNA mimics led to significant decrease in KLC2 mRNA and protein levels in HuH7 cells when compared to control cells. Additionally, siRNA-mediated knockdown of KLC2 revealed reduced cell viability of liver cancer cell lines compared to scramble siRNA transfected cells; in line with this similar results were observed in LINC00152-deficient cell lines. Furthermore, KLC2 expression was upregulated in human HCCs compared to non-tumor liver tissues in the TCGA sample cohort and was significantly associated with LINC00152 expression, thereby independently validating our human findings.

In summary, our data demonstrate that LINC00152 drives a ceRNA network in human HCC, in which KCL2 is upregulated via sponging and thus reducing the bioavaibility of miR-143-3p, which promotes proliferation of HCC cells.

4.8 A genetic variant in toll-like receptor 5 is linked to chemokine levels and hepatocellular carcinoma in steatohepatitis

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Question Bacterial translocation from the intestine is an important mechanism of liver disease progression. In contrast to toll-like rececptor (TLR) 4, the receptor for lipopolysaccharide, the receptor for flagellin, another frequent component of Gram-negative bacteria, TLR5, has rarely been studied. We wondered whether functional genetic variants in *TLR5* affect the risk to develop hepatocellular carcinoma (HCC) in patient with cirrhosis due to alcohol-associated and nonalcoholic steatohepatitis.

Methods Healthy controls (n=212), patients with alcohol abuse without liver disease (n=382), and three cohorts of patients with cirrhosis due to alcohol-associated (n=372 and n=355) and non-alcoholic steatohepatitis (NASH) (n=145), including 79, 132 and 62 patients with HCC, respectively, were genotyped for the *TLR5* rs5744174 and rs5744168 polymorphisms. Chemokine levels were measured by ELISA in patients" sera and supernatants of flagellin-stimulated healthy monocytes.

Results Frequency of the *TLR5* rs5744174 TT genotype was similar in healthy controls (33 %), controls with alcohol abuse (34 %), and patients with alcohol-associated cirrhosis in the discovery (28 %), validation (33 %) and NASH cohort (31 %). However, the TT genotype was enriched in cirrhotic patients with HCC compared to patients without HCC in the discovery, validation and NASH cohort (41 % vs 25 %; 39 % vs 29 %; 40 % vs 24 %; $p < 0.05$ each). The TT genotype remained a risk factor for HCC (OR = 1.9; CI 1.2 – 3.1; p = 0.01) after multivariate correction for age, gender, diabetes, and carriage of the *PNPLA3* 148M variant. In presence of the TT genotype, interleukin-8 induction in monocytes from healthy controls and serum levels of interleukin-8 and of CXCL1 from cirrhotic patients were significantly increased. Genetic variance of the *TLR5* rs5744168 polymorphism was not linked to the occurrence of HCC.

Conclusion The proinflammatroy TT genotype of the *TLR5* rs5744174 polymorphism is associated with an increased risk for for HCC in cirrhosis due to steatohepatitis.

4.9 Inhibition of the IL-1R1 pathway in hepatocytes reduces tumor growth in a mouse model of NAFLD-associated HCC

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Question Non-alcoholic fatty liver disease (NAFLD) is a relevant risk factor for developing hepatocellular carcinoma (HCC) even in the absence of advanced fibrosis or cirrhosis; however, little is known about the specific molecular mechanisms involved. Given the critical role of interleukin (IL)-1 signaling in inflammation and metabolism, we used hepatocyte-specific IL-1 receptor type 1 (IL-1R1) knockout (*Il1r1*$^{Hep-/-}$) mice to examine the contribution of this pathway in NAFLD-associated hepatocarcinogenesis.

Methods Diethylnitrosamine (DEN, 25 mg/kg) was given i.p. to 2-week-old, male *Il1r1*$^{Hep-/-}$ mice and wild-type (WT) littermates. From 6 weeks of age the mice were fed either a high-fat, high-carbohydrate diet (HFD, n=8/genotype) or a control diet (CD, n=4/genotype) until sacrifice at 24 weeks of age.

Results In contrast to the DEN/CD groups with a lean phenotype, all mice treated with DEN/HFD displayed significant obesity, hyperlipidemia, and hyperglycemia; however *Il1r1*$^{Hep-/-}$ mice were less susceptible to elevations of fasting glucose and HOMA-IR levels compared to WT mice. Both genotypes were similar prone to develop HFD-induced liver enlargement, elevated serum transaminases, and NAFL-histology with macrovesicular steatosis, but without significant inflammation or fibrosis. Additionally, adding HFD to DEN led to a significant increase in macroscopically visible dysplastic lesions in liver tissue compared to the CD. Despite heterogeneity, the average number and volume of tumors formed in *Il1r1*$^{Hep-/-}$ livers were lower than those for WT mice, two of which developed 10mm tumor nodules. Also histopathological examination of the left lateral liver lobe revealed a 2.7-fold increase in dysplastic foci >1mm in WT compared to transgenic mice. While both DEN/HFD groups exhibited a comparable increase in circulating MCP-1 levels and relative number of GR1-$^{+}$F4/80^{+} intrahepatic cells in parallel to a slight decrease of CD4^{+} T cells, increases in serum CXCL-1 were less pronounced in *Il1r1*$^{Hep-/-}$ mice compared to WT littermates.

Conclusions HFD-feeding to DEN-injected mice strongly enhances liver tumor development with an incidence rate of 100 % at already 24 weeks of age. There is early evidence that inhibition of the IL-1R1 pathway in hepatocytes leads to reduced tumor growth under these conditions. It remains to be determined whether improved insulin sensitivity and/or inflammatory processes in *Il1r1*$^{Hep-/-}$ mice are critical for reduced tumor burden.

4.10 The common and mutual exclusive interactome of the oncogenes YAP and TAZ in liver cancer

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The Hippo pathways effectors *yes-associated protein* (YAP) and *WW domain containing transcription regulator 1* (WWTR1; syn: TAZ) play an important role in tumor development and progression. Recent findings illustrate that both oncogenes facilitate their pro-tumorigenic properties in liver cancer *via* common and exclusive mechanisms. However, the underlying molecular network of YAP- and TAZ-induced tumorigenesis is not fully understood. Here, we aim to dissect their respective interactome in liver cancer cells.

We generated HCC cell lines expressing inducible YAP or TAZ tagged with the biotin ligase BirA, which allows labelling of potential binding partners. After biotin administration for 18 hours, biotin-tagged proteins from 4 technical replicates were pulled-down by streptavidin. Purified proteins were seperated by SDS-PAGE and submitted to tryptic in-gel digestion. The LC/MS analysis was performed using an Easy-nLC 1200 coupled to an Orbitrap Exploris 480 MS. The resulting data was analyzed using MaxQuant and Perseus software. Only proteins with a fold change >2 and a false discovery rate <0.05 compared to the control were considered for further analyses. CoIP was performed for candidate validation.

According to the selection criteria, 267 potential YAP and/or TAZ binding partners were identified among them many already known interaction factors such as transcriptions factors (TEAD family), Hippo pathway constituents (e.g. LATS) and cell junction proteins (e.g. AMOT). With 247 proteins the identified YAP interactome was bigger than the TAZ interactome with 108 potential binding partners. Interestingly, most predicted TAZ binding partner (81 %; 88/108) also interact with YAP. In contrast, for YAP the majority of binding partners was not recognized by TAZ (64 %, 159/247). STRING database analysis revealed involvement of the YAP/TAZ interactome in several cancer cell-relevant processes like chromatin remodeling, proliferation, cell polarity and actin dynamics. Remarkably the YAP exclusive interactome contains a vast amount of proteins involved in cytoskeletal remodeling and cell junctions. CoIP experiments confirmed binding of YAP with so far unkown interaction partners such as *vasodilator stimulated phosphoprotein* (VASP), which supports actin polymerization.

On the molecular level the majority of the TAZ interactome is also recognized by YAP in HCC cells. Thus, YAP may facilitate additional functions via interactions with YAP-exclusive binding partners.

4.11 Implications of NOTCH3 expression and signaling for cholangiocarcinogenesis

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Question The NOTCH pathway is an evolutionary conserved signaling pathway, which has a pivotal role for physiological liver development and regeneration. Persistent hyperactivation of NOTCH signaling is a potential driver of liver cancer development and progression, particularly of the cholangiocarcinoma (CCA) subtype. However, compared to the well-studied NOTCH1 receptor, the role of NOTCH3 is poorly characterized. Similarly, the expression of NOTCH receptors in benign precursor lesions including biliary intraepithelial neoplasia (BilIN) or intraductal papillary neoplasms of the bile duct (IPNB) was not yet investigated. Here, we elucidated the function of the atypical NOTCH3 receptor during cholangiocarcinogenesis.

Methods Gene expression analysis was performed to quantify the mRNA levels of normal bile duct epithelium, precursor lesions and invasive CCA in a matched design using a well-characterized human CCA cohort. Tissue microarray analysis was conducted to determine the expression, localization and activity levels of the NOTCH receptors on protein level in human CCA specimens. The activity and function of NOTCH1 and NOTCH3 were investigated *in vitro* in CCA cell lines. To study the impact on liver tumorigenesis *in vivo*, hydrodynamic tail vein injection (HDTV) of mice was utilized to stably overexpress hyperactive NOTCH1 or NOTCH3 in combination with an AKT oncogene in hepatocytes.

Results Analyzing patient data, we detected a gradual upregulation of NOTCH3 expression during cholangiocarcinogenesis, while the mRNA levels of the NOTCH1 and NOTCH2 receptor were not significantly or gradually altered. Analyzing protein abundance, a subgroup of CCA patients with active nuclear NOTCH1 and NOTCH3 staining were identified. Accordingly, *in vitro* analysis showed that NOTCH1 and NOTCH3 were differentially expressed among multiple human CCA cell lines. In addition, *in vivo* experiments demonstrated that NOTCH3 has a similarly potent oncogenic function relative to NOTCH1, leading to tumor formation 7 weeks after HDTV when combined with AKT overexpression. By immunohistochemistry, all NOTCH/AKT tumors were classified as CCA subtype.

Conclusions Our data suggest that particularly NOTCH1 and NOTCH3 signaling might be implicated in CCA development. Investigating the differences of both receptors on downstream signaling and tumor features is subject of our current research.

4.12 Integrative analysis reveals early and distinct genetic and epigenetic changes in intraductal cholangiocarcinogenesis

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Question A detailed understanding of the molecular alterations in different forms of cholangiocarcinogenesis is crucial for a better understanding of cholangiocarcinoma (CCA) and may pave the way to early diagnosis and better treatment options.

Methods We analyzed a clinicopathologically well-characterized patient cohort (N=54) with high-grade intraductal papillary (IPNB) or tubulopapillary (ITPN) precursor lesions of the biliary tract and correlated the results with an independent non-IPNB/ITPN associated CCA cohort (N=294). The triplet sample set of non-neoplastic biliary epithelium, precursor, and invasive CCA was analyzed by next generation sequencing, DNA copy number and genome-wide methylation profiling.

Results Patients with invasive CCA arising from IPNB/ITPN had better prognosis than CCA patients without IPNB/ITPN. ITPN localized mostly intrahepatic, whereas IPNB was mostly of extrahepatic origin. IPNB/ITPN were equally associated with small and large duct type intrahepatic CCA. IPNB exhibited mutational profiles of perihilar and distal CCA, while ITPN had significantly fewer mutations. Most mutations were shared between precursor lesions and corresponding invasive CCA but ROBO2 mutations occurred exclusively in invasive CCA and CTNNB1 mutations were mainly present in precursor lesions. In addition, IPNB and ITPN differed in their DNA methylation profiles and analyses of latent methylation components suggested that IPNB and ITPN may have different cells-of-origin.

Conclusions Integrative analysis revealed that IPNB and ITPN harbor distinct early genetic alterations, IPNB are enriched in mutations typical for extrahepatic CCA, whereas ITPN exhibited few genetic alterations and showed distinct epigenetic profiles, and IPNB/ITPN may represent a distinctive, intermediate form of intra- and extrahepatic cholangiocarcinogenesis.

4.13 Targeting cancer stem cells and YAP signaling as an effective approach to overcome sorafenib resistance in HCC

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Introduction Inhibition of neo-angiogenesis is an effective treatment strategy for advanced HCC. However, development of chemoresistance is observed in the majority of patients. Evidence suggests that cancer stem cells (CSCs) may contribute to the acquisition of resistance in many solid tumors, but their exact role in this process for HCC remains to be defined.

Aim Here, we evaluate the importance of CSCs in the development of resistance and relapse formation after exposure to sorafenib in HCC and define concomitant adaptive molecular changes.

Methods Four HCC cell lines and two primary HCC isolates were exposed to sorafenib for a total of 14 days. The treatment effects on CSCs were estimated by sphere forming capacity *in vitro* and tumor-initiating potential *in vivo*, as well as the side-population (SP) approach. Expression of CSC marker EpCAM was assessed by flow cytometry. Whole transcriptome analyses were performed across cell lines and identified potential targets, which were further validated by western blot and administration of specific inhibitors *in vitro,* as well as in authentic HCC patients.

Results Treatment effectively reduced oncogenic properties in all investigated HCC cells. However, sustained anti-proliferative effect after treatment was observed in three cell lines, while initial treatment effect in other lines was subsequently followed by rapid cellular re-growth. Anti-oncogenic effects in sensitive cell lines were associated with significant reduction in sphere forming and tumor-initiating capacity, number of EpCAM and SP cells, while resistant cells showed transient increased in CSC properties. Adaptive molecular changes developed during acquisition of resistance involved signaling pathways associated to cell survival, proliferation and cell cycle (RAS, AKT, MYC, P53). Furthermore, resistant cell lines showed compensatory upregulation of key oncogenic molecules such as YAP. Combined treatment with sorafenib and specific YAP inhibitor showed beneficial effects in resistant cell lines. Conclusively, IHC and GSEA revealed YAP enrichment in patients resistant to sorafenib treatment and in a group with high content of CSC.

Conclusion Our model recapitulates features of drug resistance observed in human HCC patients. Resistance to sorafenib might be fueled by transient expansion of CSCs. Therefore, specific targeting of CSCs as well as compensatory signaling pathways might be an effective therapeutic strategy to overcome resistance in HCC.

4.14 Overexpression of CTNNA1 supports nuclear enrichment of the oncogene YAP in hepatocarcinogenesis

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Background Hepatocytes are highly polarized cells and their physical contact with adjacent parenchymal cells is crucial to maintain liver homeostasis. Interestingly, changes of cell-cell contact and junctional structures are frequently observed in human hepatocellular carcinoma (HCC), however, how dysfunctional cellular interaction contributes to tumor formation and progression is poorly understood. In this study, we aim to identify junctional proteins that are dysregulated in liver cancer and that control activity of the oncogene yes-associated protein (YAP).

Methods Transcriptome expression data from human HCC patients (e.g. The Cancer Genome Atlas) was used to screen for dysregulated junctional factors in HCC tissues.1 For the same cohort, genomic gains and losses were analyzed. Candidates were confirmed by immunohistochemistry (tissue microarrays with 744 samples). RNAinterference was used for the genetic knock-down of genes in different liver cancer cells lines (Hep3B, HLF and HepG2) followed by functional analyses (MTT-, BrdU- and apoptosis assay). Protein binding studies were performed using co-immunoprecipitation (co-IP).

Results The adherens junction factor α-catenin (CTNNA1) was overexpressed in HCCs compared to normal/adjacent liver tissues with highest cytoplasmic expression in poorly differentiated tumors ($r=0.29$, $p\leq0.01$). Elevated CTNNA1 levels statistically correlated with poor clinical outcome ($p\leq0.05$). Interestingly, only cytoplasmic but not membranous CTNNA1 localization significantly associated with nuclear YAP levels. CTNNA1 gene amplification was rarely detected in human HCCs and therefore couldn't explain its overexpression. CTNNA1 was mainly expressed at the membrane of Hep3B cells, while it predominantly showed cytoplasmic enrichment in HLF and HepG2 cells. CTNNA1 depletion decreased HCC cell viability and proliferation but had no obvious effect on cell apoptosis. CTNNA1 silencing led to reduced YAP protein but not transcript levels in HCC cells. Lastly, co-IP experiments revealed no direct interaction between CTNNA1 and YAP.

Conclusion CTNNA1 overexpression in HCC cells supports cell proliferation probably *via* indirect induction/stabilization of YAP. Currently, proteomics analysis after CTNNA1 co-IP aims to identify the mechanistic link between CTNNA1 and high nuclear YAP enrichment (BioID technology).

Reference [1] Cancer Genome Atlas Research Network. Comprehensive and Integrative Genomic Characterization of Hepatocellular Carcinoma. Cell. 2017;169(7):1327–1341 e1323.

4.15 Mathematical modeling of YAP and TAZ nuclear/cytoplasmic shuttling in liver cancer cells

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Background YAP (*yes-associated protein)* and TAZ (*WW domain containing transcription regulator 1, WWTR1)* are oncogenic transcriptional co-activators, which facilitate biological functions of the Hippo pathway in many tumor cells. However, if and to which extent YAP and TAZ differentially respond to extracellular information such as cell-cell contact in the liver carcinogenesis is not well understood.

Methods Using lentiviruses, we stably transfected Hep3B (hepatocellular carcinoma; HCC) cells with Cerulean-tagged histon 2B (H2B), Venus-tagged YAP and mCherry-tagged TAZ reporter constructs that allowed us to study nuclear-cytoplasmic shuttling of both factors via time-lapse microscopy. Cells were cultured under varying cell density conditions and time-resolved data was analyzed in a quantitative manner with the software Fiji. To address the question, whether the differential YAP and TAZ shuttling can be explained with dynamic differences in nuclear transport kinetics, we created a *partial differential equation* (PDE)-based computational model using a Spatial Model Editor. The PDE model simulates the two-dimensional reaction-diffusion equation, which takes into account the concentration of species at a position in time and the diffusion constant of species, and the reaction term, which describes rate of change of species concentration at every point inside the compartment.

Results As expected, YAP and TAZ shuttled out of the nucleus upon increased cell density; however, dynamics between both factors differed with YAP already responding at lower densities than TAZ. The interactions between phosphorylated (cytoplasmic) as well as unphosphorylated (nuclear) YAP and TAZ, their degradation and how these processes impact the molecular shuttling over the nuclear membrane was mathematically modeled. With help of the PDE model we could describe experimental observations that the dynamics of TAZ is considerably different from YAP, moreover, we could pinpoint key parameters, which define these differences in subcellular localization.

Summary We developed an analysis tool and a novel PDE-based mathematical model that quantitatively describe the cellular YAP/TAZ response of HCC cells on varying cell density conditions. As several YAP and TAZ-specific inhibitors are currently being developed (AACR virtual annual meeting; June 22-24, 2020), there is a growing importance of exploring how strongly YAP and/or TAZ respond to nuclear exclusion on these substances.

4.16 Dissecting molecular drivers of sorafenib resistance in HCC: integrative genomic analyses of poor and good responders

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Introduction Sorafenib was the only approved systemic therapy with demonstrated survival benefit in advanced stage of hepatocellular carcinoma (HCC) for more than a decade. Despite the current success of the multi-tyrosine-kinase inhibitor sorafenib, many of the patients respond poorly to the drug treatment or relapse quickly after initial remission.

Aim The aim of this project was to identify drivers of the sorafenib resistance in the group of HCC patients with the worst response to the treatment.

Methods To identify novel predictive markers of sorafenib response we performed integrative RNA sequencing and whole-exome sequencing analyses. Based on our cohort of 19 HCC patients, we first identified two specific sorafenib-treated subgroups of patients, i.e. long-term responders (n=12) (best) and primary non-responsers (n=7) (worst). Potential drivers of drug resistance were evaluated by Ingenuity Pathway Analysis (IPA) and Gene Set Enrichment Analysis (GSEA). Validation was performed in our *in vitro* model of sorafenib resistance by western blot.

Results Patients with worst response were characterized by significantly shorter treatment duration and poor overall survival than good responders (66,6 months and 133,3 months respectively; p<0,0004). Molecular analyses revealed that poor responder group was associated with activation of pathways commonly linked to proliferation, oxidative stress and inflammation. Furthermore, genes sets associated with activation of Pi3K/AKT/mTOR, NFkB as well as YAP and Hippo signaling were identified to be significantly enriched in this subgroup whereas growth factor signaling as well as KRAS signaling characterized good responders. Importantly, hypoxia of the surrounding tumor microenvironment was significantly enhanced in worst responders. From hypoxia-related targets, we could observe that proteins from the 14-3-3 family (7 isoforms) might play a significant role in acquisition of resistance.

Conclusion Our integrative analyses delineate distinct molecular alterations responsible for response to sorafenib in HCC. Defining the actionable targets of resistance and subsequent inhibition, e.g. components of 14-3-3 protein family, might facilitate to improve the systemic therapy of HCC and is currently under way.

4.17 Deciphering the role of FHL1 as tumor suppressor in gallbladder cancer

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Question Gallbladder cancer (GBC) is an aggressive malignancy of the gallbladder. Although it belongs to a rare disease, its incidence is rising and comprising the most common biliary tract cancer (BTC). Its molecular prognostic markers have not yet been identified and only 10 % of patients are suitable for potentially curative surgery. Therefore, a better understanding of the molecular mechanisms of GBC oncogenesis and the identification of novel potential targets for GBC therapy are urgently needed.

Methods This project is the first effort to elucidate the proteome of GBC patients and aims at characterizing the molecular function of the potential tumor suppressor genes in GBC. Quantitative mass spectrometry from Formalin-Fixed Paraffin-Embedded (FFPE) tissue of 5 GBC samples and 5 healthy gallbladder tissue samples were performed to analyse deregulated proteins in GBC. In total 4,827 proteins were detected of which 1,766 were significantly deregulated (adjusted p-val ≤ 0.05). Among these, 676 proteins were significantly downregulated and 1,090 proteins were upregulated. One of the deregulated proteins, Four and a half LIM Domains 1 (FHL1) is significantly downregulated (log2 fold change= -2.8, p-val ≤ 0.001). GBC cell lines with

stable and inducible expression of FHL1 were generated to decipher the role of FHL1 in GBC.

Results Functional analysis *in vitro* showed that FHL1 significantly inhibits GBC cell lines proliferation and colony formation, while increases cell adhesion ability. Furthermore, molecular signaling pathway analysis demonstrated that FHL1 is linked to NOTCH pathway. Based on qPCR, FHL1 overexpression significantly downregulates transcription of typical N1ICD target genes HES1, HES4, HES7, HEY1 and c-Myc. Correspondingly, dual-reporter luciferase assay confirmed the inhibition of active N1ICD-driven transcription by overexpressing FHL1 in GBC cell lines.

Conclusion Thus, this present study underlines the important tumor-suppressive roles of FHL1 in GBC and highlights the potential role of FHL1 in NOTCH signalling.

4.18 Radiofrequency ablation compared with microwave ablation for the treatment of liver cancer: a meta-analysis.

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Question Radiofrequency ablation (RFA) and microwave ablation (MWA) are the main ablative methods utilized in the treatment of hepatic lesions. Although efficacy of RFA has been evaluated in several studies, effectiveness of MWA and comparison between the two approaches remain unclear. Aim of the survey is to compare MWA with RFA in the treatment of liver cancer.

Methods The systematic review and meta-analysis were conducted according to the PRISMA guidelines. A systematic search of MEDLINE (PubMed and Ovid) and the Cochrane Central Register of Controlled Trials was conducted for studies published from 2010 onwards. For all outcomes of interest, meta-analyses for the Odds Ratio (OR) have been performed. A random-effects model was used for the meta-analyses. Complete ablation (CA), local tumor progression (LTP), intrahepatic distant recurrence (IDR), and complications were analyzed.

Results Fifteen studies with a total of 2169 patients were included in our analysis. The meta-analysis found non-significant difference in CA rates between MWA and RFA (OR, 1.10; 95 % CI, 0.78 – 1.55; p = 0.5898). Specifically, for the four randomized clinical trials (RCTs), meta-analysis outcomes remained consistent with the main overall results (OR, 1.28; CI, 0.54 – 3.05; p = 0.5706). Rates of LTP were comparable between MWA and RFA (OR, 0.79; 95 % CI, 0.53 – 1.20; p = 0.2689). In the subgroup analysis, which included only RCTs, significantly reduced rates of LTP were demonstrated in the MWA group compared to RFA (OR, 0.40; 95 % CI, 0.18 – 0.92; p = 0.03). Meta-analysis showed no significant differences in IDR between MWA and RFA (OR, 0.73; 95 % CI, 0.45 – 1.16; p = 0.1826). The risk of major complications was not different between the two approaches (OR, 0.80; 95 % CI, 0.46 – 1.37; p= 0.4129). No significant differences were found between the two modalities in patients with tumors less or larger than 3 cm. A further subgroup meta-analysis was conducted to evaluate the effectiveness of ablation methods in patients with hepatocellular cancer and colorectal liver metastases separately. Although the majority of outcomes were similar between the two approaches, subgroup analysis of included RCTs showed statistically decreased rates of LTP in patients with HCC following MWA compared to RFA.

Conclusions MWA showed promising results and demonstrated better outcomes in terms of LTP compared to RFA. Further randomized trials are required to evaluate the superiority of MWA over RFA.

4.19 Dissecting the molecular interplay between tumor cells and –stroma in primary liver cancer

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Hepatocellular carcinoma (HCC) and cholangiocarcinoma (CCA) constitute the two most prevalent types of primary liver cancer, together accounting for approximately 95 % of cases. In contrast to the desmoplastic appearance of most CCAs, the majority of HCCs usually appear as stroma-scant tumors. Still, cases with unusual phenotypes such as solid CCA and sclerotic HCC can be detected. However, the tumor microenvironment which consists of cancer-associated fibroblasts, endothelial cells, various types of immune cells, and other stroma elements may play a key role in cancer development, progression and may even contribute to the process of determining the cancer phenotype. On the basis of these observations, this project aims at elucidating the mechanism driving the tumor phenotype of primary liver cancer in order to identify novel therapeutic targets and eventually predictive biomarkers. For this purpose, each five informative samples of CCA and HCC with a stroma-poor or –rich phenotype, respectively, were selected for exome and transcriptome sequencing. Data integration revealed 29 genes potentially driving the tumor phenotype. To validate the loss-of-function candidates, two transposon-based mosaic mouse models were used (HCC model: MYC-AKT1 in wildtype C57BL/6 mice; iCCA model: KRASG12V in p19-/- mice). Transposon vectors together with the shRNA library (5 shRNAs/per candidate gene) were hydrodynamically injected into the tail vein of mice. Tumor development was monitored and all dissectible tumor nodules were individually evaluated by histological analysis. In the case of a switch in tumor phenotype (change of tumor type or alteration of tumor stroma), the expressed shRNA was identified by sequencing. Single gene validation and further mechanistic analyses are currently ongoing and the results will be presented during the meeting.

4.20 Genetic variants of interleukin 1 beta are associated with viral hepatitis-related HCC in Caucasian patients

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Background The proinflammatory cytokine interleukin 1 beta (IL-1b) mediates several immune responses and promotes the development of liver cirrhosis and hepatocellular carcinoma (HCC). Alterations in the IL-1b gene can affect cytokine expression and signaling and can augment the progression of liver disease towards more severe stages. We aimed to investigate the association of polymorphisms within the promotor region of the IL-1b gene with liver disease progression in Caucasian patients related to chronic infection with hepatitis B virus (HBV) and hepatitis C virus (HCV).

Method In this retrospective study, 632 Caucasian patients with chronic HBV infection, of whom 105 patients were diagnosed with liver cirrhosis and 64 with liver cirrhosis and HCC, 101 Caucasian patients with chronic HCV infection and HCC and 124 matched HCV controls without HCC were enrolled. Host genomic DNA was extracted from peripheral blood samples. Genotyping of *IL-1b* polymorphisms rs1143623, rs1146327 and rs16944 was performed.

Results The frequencies of *Il-1b* rs1146327 TT and rs16944 CC were higher in patients with HBV-related HCC than in patients without HCC (rs1146327 TT: 48 % vs. 33 %, p=0.018, rs16944 CC: 47 % vs. 31 %, p=0.001). The same was true in patients with HCV-related HCC and the matched controls without HCC (rs1146327 TT: 53 % vs. 39 %, p=0.032, rs16944 CC: 53 % vs. 40 %, p=0.031). In multivariate analysis, only the IL-1b rs16944 CC genotype remained independently associated with HCC in patients with chronic HBV infection (OR=3.78 [95 % CI: 1.08-13.27] p=0.038) and in patients with chronic HCV infection (OR=1.80 [95 % CI: 1.01-3.21] p=0.048). In haplotype analysis, the haplotype including both variants rs1143627 TT and rs16944 CC was a risk factor for the development of HBV-related HCC (OR=1.55 [95 % CI: 1.04-2.32] p=0.033) and HCV-related HCC (OR=1.66 [95 % CI: 1.09-2.53] p=0.017).

Conclusions We identified an association of common *IL-1b* polymorphisms with the presence of HBV- and HCV-related HCC in a Caucasian population. It seems that the alterations within the *IL-1b* gene are shared risk factors for the development of HCC related to viral hepatitis. Further studies in larger cohorts and functional analyses are required to confirm the results and to elucidate the role of the risk loci in liver fibrogenesis and carcinogenesis.

4.21 Establishment of a transgenic, single-nodule model of liver cancer in a fibrotic background

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The majority of Hepatocellular Carcinomas (HCC) evolves from fibrotic liver parenchyma. So far, there is no experimental model of liver cancer in the background of fibrosis to study intratumoral application of immunotherapies and their impact on disease recurrence after tumor resection. Therefore, the aim of our study was to establish a clinically relevant murine model of single-nodule liver cancer in fibrotic livers facilitating investigations on (neo)adjuvant therapies.

For this purpose, we wanted to apply our established method for local tumor induction in mice by intrahepatic electroporation of oncogenic transposons in the context of liver fibrosis mediated by CCl4-intoxication. At an age of 6-8 weeks, C57BL/6-p53fl/fl mice were electroporated with oncogenic transposons for KRasG12V and an expression plasmid for Cre recombinase. CCl4, resolved in mineral oil was applied twice weekly for a total duration of 4-6 weeks starting before or after electroporation. Tumor development was monitored, liver specimen were investigated by HE- and Sirius-Red-staining, and degree of fibrosis was determined.

Three experimental groups of mice were used. In the first two groups CCl4 injections were started 2 or 3 weeks prior to electroporation whereas in the third group fibrosis induction was started immediately after electroporation. We found that CCl4 treatments for induction of fibrosis before electroporation did not interfere with successful tumor induction. In all groups, tumors were histologically verified in explanted tissue specimen. Liver fibrosis could also be confirmed in all three groups. However, mice starting with CCl4-treatments after electroporation developed only a low to moderate degree of fibrosis before tumor size became critical. In contrast, mice which received the first CCl4 treatments prior to tumor induction showed an average to high grade fibrosis in both groups according to the Desmet/Scheuer staging score once tumors reached a palpable/injectable size. Nevertheless, when applying the longer period of CCl4 injection prior to electroporation the reliability of tumor induction might be negatively affected.

In summary, we have developed an experimental murine tumor model that enables studies on intratumoral application of oncolytic virotherapy in the presence of coexisting fibrosis and its effects on the tumor microenvironment, thereby reflecting the clinical situation.

4.22 Dimethyl fumarate (DMF) inhibits proliferation and induce specific cell death in hepatocellular carcinoma (HCC)

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Introduction We could show that dimethyl fumarate (DMF) treatment leads to induction of apoptosis in NF-KB-dependent tumors. In addition, a mouse xerograph model revealed that DMF application led to inhibition of metastasis formation. The inhibition of metastasis formation was NF-κB-independent. Thus, we analyzed NF-KB-independent tumor cell lines (*e.g.* from colon cancer, pancreatic cancer) regarding migration and invasiveness. DMF reduced these parameters important for metastasis formation in cell lines of these tumors. Here, we analyzed whether DMF application leads to an inhibition of proliferation and cell death induction in hepatocellular carcinoma (HCC) cell lines.

Methods Human HCC cell lines Hep3B, HepG2 and Huh7 were treated with DMF (from 25µM up to 100µM) for up to 72h. The cellular ATP content was measured using a luminescence based assay. To test the effects on proliferation cells were either treated with different DMF concentrations or treated with DMF and 50µM zVAD a caspase inhibitor to prevent induction of apoptosis. HCC cell lines were stained with Cytopainter Cell Proliferation Staining Reagent. The proliferation of the HCC cell lines were monitored up to 96h by flow cytometry. Cell death induction was analyzed by flow cytometry.

Results DMF application resulted in a time and dose-dependent energy (ATP) depletion in all cell lines used. We analyzed whether the decrease in ATP level induces apoptosis or inhibition of proliferation. Interestingly, the DMF treatment resulted in a moderate cell death induction, which was time and dose-dependent. To investigate whether proliferation is effected cells were treated with DMF in combination with zVAD, a caspase inhibitor, to prevent cell death. Accordingly, to the ATP-depletion HCC cell lines showed a strongly diminished proliferation rate.

Conclusion Here, we could show that DMF is capable to inhibit proliferation and induce specific cell death in HCC cell lines. DMF is already clinically approved for treatment of multiples sclerosis and displays only minor side effects. Hence, targeting cellular metabolism by substances like DMF should be regarded as a promising approach to develop novel therapeutic tools to treat solid tumors such as HCC to prevent metastasis formation and tumor growth.

4.23 Upcoming expression of ALK1 in HCC determines the switch from tumour-suppressive to tumour-promoting BMP-9 signalling

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Question Bone morphogenetic protein (BMP)-9 is a hepatic cytokine that belongs to the TGF-β superfamily. Recent studies have shown that the role of BMP-9 in hepatocellular carcinoma (HCC) is rather controversial. BMP-9 was

described to promote cell proliferation and epithelial-to-mesenchymal transition (EMT) in some studies, whereas in others it acted anti-proliferative and decreased mesenchymal markers in HCC cells. HCC is still the third leading cause of cancer death worldwide and new therapeutic targets to improve therapeutic strategies are urgently needed. Aim of this study was therefore to better understand BMP-9 signalling in HCC.

Methods Public databases such as The Cancer Genome Atlas (TCGA) and The Cancer Proteome Atlas (TCPA) were used to extensively analyse available expression data for BMP-9 signalling pathway components. Expression of ALK1, ALK2, Activin A Receptor Type 2A (ACVR2A), Activin A Receptor Type 2B (ACVR2B), BMPRII (BMPR2), endoglin (ENG), Smad1 (SMAD1) and others were assessed. Smad-1-phosphorylation, cell -proliferation and -migration upon BMP-9 stimulation were determined in two human HCC cell lines (HLE and Hep3B) by Western blot, real-time PCR, proliferation- and wound closure assays. Freshly collected samples from 6 HCC patients were additionally analysed for validation of the *in silico* findings.

Results *In silico* results show that Alk1 is upregulated in HCC patient samples and that presence of Alk1 is associated with attributes of cancer progression. In line with this, BMP-9 induced proliferation, migration and EMT in HLE cells, which display high levels of Alk1, but not in epithelial Hep3B cells with low Alk1 expression. Over-expression of Alk1 in Hep3B could partially induce tumorigenice effects. Finally, we show that Alk1 expression leads to decreased Smad-1 phosphorylation and an enhanced tumour-promoting expression pattern, whereas non-Alk1 BMP-9 signalling (e.g. via Alk2) uses the canonical Smad-1-pathway for tumour-suppressive signalling.

Conclusions These results suggest that in tumour cells presence of Alk1 promotes tumour progression whereas non-malignant hepatocytes (and some HCC cell lines) do not express Alk1 and respond to BMP-9 via the Smad-1 pathway, thereby stabilizing the differentiated (non-proliferative) parenchymal phenotype and acting tumour-suppressive. Thus, individualized determination of Alk1 expression in HCC patients should help to predict potential responsiveness to BMP-9 targeted therapies.

4.24 Expression and function of histone deacetylase 7 in hepatocellular carcinoma

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Introduction An imbalance between the acetylation and deacetylation of histone proteins regulated by histone deacetylases (HDACs) has been shown in different types of cancer including hepatocellular carcinoma (HCC). HDAC inhibition appears as promising therapeutic strategy for HCC. However, mostly unspecific HDAC inhibitiors have been applied and the knowledge about the expression and role of individual HDACs in HCC is very limited.

The aim of the study was to unveil the expression and function of HDAC7 in HCC.

Methods and Results HDAC7 expression was significantly increased in 4 human HCC cell lines (Hep3B, HepG2, PLC, Huh7) compared to primary human hepatocytes (PHH). Furthermore, HDAC7 expression in a spontaneous murine HCC model and human HCC specimens compared to non-tumorous liver tissue were significantly increased. TCGA (The Cancer Genome Atlas) data set analysis revealed a significant correlation between high HDAC7 expression and poor patients' survival. Interestingly, in silico analysis applying the GEPIA-platform showed that HDAC7 expression in human HCC tissues correlates significantly with the expression of genes regulated by hypoxia such as GLUT1 and VEGF. In line with this, hypoxia significantly increased HDAC7 expression in human HCC cells in vitro. Further in vitro studies showed that siRNA mediated depletion of HDAC7 significantly reduced NFkB-activity in HCC cells. Furthermore, we found increased p62 and LC3 II protein levels in HDAC7-depleted cells indicating an impairment of the autophagic flux.

Summary and Conclusion Our study indicates HDAC7 as strong pro-tumorigenic factor in HCC and newly identifies hypoxia, a condition frequently occurring in HCC, as promotor of HDAC7 expression in HCC. These data indicate HDAC7 expression as prognostic marker and suggest specific HDAC7 inhibition as promising therapeutic strategy in HCC.

4.25 Combining targeted therapies with HDAC inhibitors to tackle the p53 signaling pathway in hepatocellular carcinoma

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Question The incidence of hepatocellular carcinoma (HCC) is increasing. Despite the progress in treatment options for HCC, drug therapies are often not sufficiently effective and associated with several side effects in patients with liver cirrhosis.

Preliminary work of our research group revealed a Mcl-1-dependent synergism of the multi-kinase inhibitor Sorafenib and the histone deacetylase inhibitor (HDACi) Vorinostat in the treatment of cutaneous T-cell lymphoma. Aim of this project was to evaluate whether such a synergistic effect can also be achieved in HCC by a combined treatment with Regorafenib and the HDACi Panobinostat. Panobinostat inhibits proliferation of malignant cells and is already approved for treatment of multiple myeloma.

Methods HepG2 and Huh7 hepatoma cells were incubated with Regorafenib (1-20 µM), with Panobinostat (5-20 nM) and with combinations of both therapeutics for 24-120 h. Cell viability was investigated by MTS assay and cell death was determined by flow cytometry after 7-AAD/FITC-Annexin V staining. Levels of p53 family were investigated by western blotting.

Results Panobinostat increased cell death induced by Regorafenib, both in HepG2 and in Huh7 cells. However, in Huh7 cells higher doses and longer incubation time were required for comparable effects. Monotherapy of HepG2 cells with Regorafenib (5µM) or Panobinostat (5 nM) led to a reduction of cell viability by 10 % after 72 h. In contrast, after combined treatment viability was reduced by 20 % after 72 h. Of note, treatment with Regorafenib alone and in combination with Panobinostat dose-dependently increased levels of p53 family proteins in HepG2. In Huh7, Regorafenib (5 µM) reduced cell viability by 20 % after 96 h. Additional treatment with Panobinostat (20 nM) led to a reduction by 40 %.

Enhancement of regorafenib-mediated cytotoxic effects by Panobinostat was shown by flow cytometry. Specific cell death of HepG2 cells after monotreatment with Regorafenib (5 µM) was about 24 % after 48 h, and about 42 % after 72 h. Combined treatment with Regorafenib (5 µM) and Panobinostat (5 nM) resulted in 41 % cell death after 48 h and 68 % after 72 h.

Conclusions Combination of the current targeted therapy Regorafenib with the HDACi Panobinostat increased the effectiveness of therapy in HepG2 and Huh7 cells. Based on these data, the long-term goal is to develop a form of therapy that allows a dose reduction of Regorafenib with the same outcome and reduced side effects.

4.26 The Alpha-1-Fetoprotein (AFP) – A novel target gene for p63

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Background Hepatocellular carcinoma (HCC) is the fifth common tumor entity worldwide. Despite advances in new technologies in both diagnosis and treatment, incidence and mortality continue to rise. So far, only orthotopic liver transplantation or surgical resection is curative. However, in many cases liver transplantation or resection of the tumor is not possible. Thus, novel treatment options are currently under investigation, *e.g.* combination treatments with checkpoint inhibitors and blockers of angiogenesis. Nevertheless, there is an urgent need for additional highly efficient approaches to treat HCC. Here, we analyze the role the Alpha-1-Fetoprotein (AFP) and its regulation by p53 family members to identify new possible therapeutic options to target HCC.

Methods *In silico* binding site analysis was done using the following databases: Transfac®, Jaspar. Hep3B cells were transfected with the following plasmids pcDNA-GFP (control), pcDNA TAp53, pcDNA TAp63, pcDNA TAp73 und pcDNA ΔNp73, respectively. mRNA was extracted using RNeasy Minikit (Qiagen). Upon reverse transcription cDNA was analyzed by quantitative PCR. Protein expression of p53 family members and AFP was analyzed by Western blot analysis.

Results Here, we show that AFP is a target protein of p53 family members. We identified *in silico* putative binding sites for p53, p63 and p73 in the promotor region and intron 1, 2, 3, and 4 of the AFP gene. Overexpression of p53, p73 and especially p63 lead to a downregulation of AFP expression on transcript (mRNA) level. In contrast, DNp73 has no influence on AFP expression. The data was verified by analysis of AFP protein expression. AFP protein levels were reduced after p53, p73 and p63 overexpression. Underlining the important role of p53 family members as negative regulators for AFP.

Conclusion Here, we demonstrate that p53 family members play an important role in regulation of AFP expression. Putative binding sites for all three family members (p53, p63 and p73) have been identified within the AFP gene. All full-length p53 family members are negative regulators for AFP expression. DNp73 did not show a regulatory effect on AFP expression. Of interest, p63 was identified as a novel negative regulator of AFP expression.

AFP is not only a tumor marker for HCC but also involved in downregulation of various immune responses. Thus, understanding the crosstalk of p53 family members and AFP expression shed new light on the pathophysiology of HCC.

4.27 Key enzymes in pyrimidine synthesis, CAD and CPS1, predict prognosis in hepatocellular carcinoma

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Background and Aims Individual patients with hepatocellular carcinoma (HCC) have a highly variable clinical course. Therefore, there is an urgent need to identify new prognostic markers to determine prognosis and select specific therapies. Recently, it has been demonstrated that dysregulation of the urea cycle (UC) is a common phenomenon in multiple types of cancer. Upon UC dysregulation, nitrogen is diverted toward the multifunctional enzyme carbamoyl-phosphate synthetase 2, aspartate transcarbamoylase, and dihydroorotase (CAD) and increases pyrimidine synthesis. In this study, we wanted to analyze the role of CAD and carbamoyl-phosphate synthetase 1 (CPS1), a rate limiting enzyme of the UC highly expressed in hepatocytes, in HCC.

Methods We created a tissue microarray to analyze expression of CAD and CPS1 by immunohistochemistry in a large and clinicopathologically well characterized cohort of HCC patients (n=561) that underwent surgery.

Results CAD was induced in recurrent HCC tumors and high expression predicted shorter overall survival. CPS1 was downregulated in HCC, was further reduced in recurrent tumors and distant metastases, and additionally, low CPS1 levels were associated with short overall survival. A combined score of both enzymes was found to be an independent prognostic marker in a multivariate cox regression model (HR 1.37, 95 % confidence interval 1.06-1.75, p=0.014).

Conclusion Two key enzymes in pyrimidine synthesis, CAD and CPS1 are dysregulated in HCC development and progression, and dysregulation is associated with short survival. Inhibition of pyrimidine synthesis may represent a novel strategy in the treatment of HCC.

4.28 Functional relevance of the tumor suppressor activity of mir-107 in liver cancer

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Background Hepatocellular carcinoma (HCC) accounts for 90 % of all primary liver cancers, and it is the third leading cause of cancer-related mortality worldwide. Treatment options are limited and survival after diagnosis is poor, as the incidence of hepatocellular carcinoma is still almost equal to its mortality rate. Previously we hypothesized that HCC development in murine models might feature more homogenous transcriptional profiles, thus leading to the discovery of novel transcriptional networks in HCC. Based on this hypothesis our research group has conceived, and recently published (*Gastroenterology 2019*), an innovative approach that starting from the integrated analyses of three different HCC mouse models has led to the discovery of previously unrecognized transcriptional networks driving hepatocarcinogenesis in human. Preliminary analysis of microarray data indicated that miR-107 is downregulated in the liver of animal models for HCC, hence suggesting a tumor suppressor role for miR-107.

Method Mouse and human HCC cell lines were transiently transfected with either miRNA mimics or siRNA (e.g., inhibition of miRNA targets), and the effect on cell survival was assessed by performing proliferation assays (MTT), cell death assays (TUNEL), and migration assays (soft agar).

Results Administration of miR-107 mimics to liver cancer cell lines caused a significant reduction in proliferation and migration capacity, and increased cell death. In accordance, a significant delay in wound healing in cells over-expressing miR-107 was detected. Unbiased genome-wide analysis identified 12 unfavorable prognostic markers, among them KIF23, was found of particular interest. Here we show that KIF23, a member of the kinesin superfamily of microtubule-based motors proteins, was found to be directly regulated by miR-107. Notably, significantly reduced migratory and invasive properties were observed after targeting cancer cell lines with KIF23 siRNAs.

Conclusion Collectively, our work lead to a more comprehensive view of the role played by miR-107 signaling in hepatocarcinogenesis. Our work supports the hypothesis that miR-107 acts as a tumor suppressor gene in the context of hepatocarcinoma. We show that KIF23, a component of the centralspindlin complex, is a novel miR-107 target gene. Due to continued progress in the field of kinesine inhibitors, KIF23 represents a promising candidate for pharmacological targeting of signaling pathways downstream of miR-107.

4.29 Survival analysis of the most frequent Single Nucleotide Variants in Hepatocellular Carcinoma

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Hepatocellular carcinoma (HCC) is the most common type of liver cancer and its incidence is rising. The introduction of new systemic therapies, including immune-based therapies and biomarker driven therapies, has improved survival in patients at advanced stages. However, overall survival is still poor, and recent advances in understanding of the molecular alterations of HCC have not translated yet into novel biomarkers. Over the past decade, major advancements in 'omic' technologies have enabled monitoring of a variety of molecular and organismal processes. A comprehensive analysis of single gene mutations in HCC might lead to detect biomarkers that improve our prognosis and treatment. We developed a bioinformatics pipeline capable of analyzing genomic data to identify key regulatory molecular changes in HCC development and their influence in patient"s prognosis. By looking at genetically determined subgroups of HCC in the TCGA Liver Cancer dataset, we managed to obtain 15 genes frequently affected by oncogenic mutations and analyzed their influence in patient"s survival, identifying CSMD1 as a prognostic biomarker candidate. Nevertheless, the validation in the ICGC HCC database showed that it did not have any statistically significant influence in overall survival. This work reveals that the most frequent single gene mutations are not enough for significant survival changes in HCC and that we should focus our efforts in integrative analysis of clinical information and multi-omics to maximize our clinical benefits in this devastating disease.

4.30 Einfluss von polyP-Substraten auf das Wachstumsverhalten des HCCs als Grundlage für ein innovatieves Tumormodell

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Einleitung Eine Herausforderung der Medizin ist eine Therapie des Krebs, der stark schwankende Charakteristika aufweist und patientenindividuell sehr unterschiedlich sein könnte. Je nach Mikromilieu des Tumors und Immunstatus kann ein Tumor in verschiedenen Ausprägungen auftreten. Bisher wird anhand der Klassifizierung mittels spezifische Biomarker die Therapiestrategie festgelegt. Von großer Bedeutung wäre eine individualisierte Strategie, die unter Berücksichtigung des individuellen Immunstatus und der Mikroumgebung des Tumors erfolgt. Für eine derartige personalisierte Analyse fehlen die entsprechenden Werkzeuge. Ziel unseres Projekts ist die Entwicklung eines personalisierten Tumormodells zur patientenindividuellen Wirkstofffindung und Anpassung der Krebstherapie. In einem ersten Schritt wurden hierzu die Kultivierung von Zelllinien in 2D-Kultur und in einem 3D-Modell sowie die Supplementierung des Zellkulturmediums mit Energieträgern wie z.B. polyPhosphat (polyP)-Substraten getestet.

Methoden Um eine mögliche Toxizität von polyP auszuschließen -wurden humanen Hep3B Tumorzellen unter klassischen 2D-Kulturbedingungen mit diesen extrazellulären Energieträgern kultiviert. Der Einfluss unterschiedlicher Medien sowie des Supplements Polyphosphat (polyP als amorphe Nanopartikel:[NatriumpolyP] und als Salz[Calcium-,Magnesium- und StrontiumpolyP]); dient als exogene Energiequelle und verhindert hypoxische Zustände) wurde auf drei Ebenen untersucht: Mittels Viabilitätsassay wurden Zellmetabolismus und Toxizität von polyP evaluiert; mittels FACS und Proliferationsassays wurden Effekte von polyP auf den Zellzyklus und die Zellproliferation untersucht. Zudem wurde getestet, ob polyP Glucose ersetzen kann.

Ergebnisse polyP hatten keinen Einfluss auf das Überleben von Hep3B-Zellen und waren nicht toxisch in der Standard-Kultur. Nach Zugabe von polyP stieg die metabolische Aktivität der Hep3B Zellen nach 4 Tagen an. Nach einer Glucosedepletion zeigte sich im MTS-Assay, dass polyP jedoch Glucose nicht als Energielieferant ersetzen kann. In Zellzyklusanalysen zeigte sich, dass nach der Zugabe von polyP Hep3B-Zellen schneller proliferieren. Der Zusatz von polyP-Substraten verbessert die metabolische Aktivität von Hep3B-Zelllinien in 2D-Kultur, ohne dabei toxisch zu wirken. PolyPs haben keinen Einfluss auf der Proliferation und Zellzyklus der Zellen, somit kann die Zugabe von polyP zu besseren Kultivierungsbedienungen zur Erforschung des Einflusses von Medikamenten schaffen.Die Ergebnisse dienen als Grundlage für weitere Untersuchungen, die klären sollen, ob eine Kultivierung primärer humaner Tumorzellen auf polyP-haltigen Scaffolds möglich ist.

Poster Visit Session V Viral Hepatitis and Immunology
Saturday, January 30, 2021, 11:00 pm – 11:45 pm, Poster Session Virtual Venue

5.4 Peritoneal cytokines of patients with decompensated liver cirrhosis drive NK and T cells towards an activated phenotype

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Question Liver cirrhosis is the end-stage of every chronic liver disease and patients with advanced cirrhosis have an increased susceptibility to infections and notoriously spontaneous bacterial peritonitis (SBP). The role of the adaptive immunity in this setting remain elusive and presently, less is known regarding the function of lymphocytes in these patients. In this study, we aim to investigate the role of NK cells and T cells in the peritoneal cavity, a common anatomical site for infections in cirrhosis.

Methods Matched peripheral blood and ascites fluid were collected from 46 patients with decompensated cirrhosis, with or without SBP. Phenotype and function of NK cells and T cells were analyzed using high-dimensional flow cytometry and obtained data were compared to blood samples of healthy controls *(n=24)* and patients with compensated cirrhosis *(n=11)*. Cytokines in matched plasma and ascites were measured using LUMINEX-based multiplex bead assay.

Results Circulating NK cells were elevated in patients with liver cirrhosis and increased even further in the peritoneal cavity in these patients. During SBP, circulating as well as peritoneal NK cell frequencies decreased despite higher proliferative capacity. In contrast, $CD8^+$ but not $CD4^+$ T cells were enriched in the peritoneal cavity whereas no differences were observed during SBP. In line with this, $CD4^+$ and $CD8^+$ T cells, as well as NK cells displayed an activated, tissue-resident phenotype in the ascites compared with circulation. This was also corroborated by increased expression of tissue-homing receptors and a higher functional avidity following stimulation with innate-like cytokines. Further, levels of pro-inflammatory cytokines were elevated in ascites fluid compared with plasma.

Conclusions Peritoneal T cells and NK cells are driven towards an activated tissue-resident phenotype and this could be explained by the distinct pro-inflammatory cytokine milieu in the peritoneal cavity.

5.5 Immunoregulatory role of the IL-33/amphiregulin axis in acute immune-mediated liver disease

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Introduction Autoimmune hepatitis is an inflammatory liver disease that is characterized by destruction of liver tissue by autoreactive T cells. Consequently, interleukin 33 (IL-33) is released from necrotic hepatocytes and binds to the IL-33 receptor ST2 thereby activating group 2 innate lymphoid cells (ILC2) and regulatory T cells (Tregs). We have previously shown that IL-33 pre-treatment ameliorated concanavalin A (ConA)-induced immune-mediated hepatitis but the underlying mechanisms remain unknown. Following activation, ILC2 and Tregs express amphiregulin (AREG). Since AREG is associated with tissue repair and immunosuppressive functions, we aimed at investigating the role of ILC2- and Treg-derived AREG in the regulation of hepatic inflammation.

Methods For induction of immune-mediated hepatitis, C57BL/6 (WT) and *Areg* -/- mice received i.v. ConA and were analysed 24 hours later. To study the effect of IL-33 on immune cell phenotype and liver disease pathology, mice received i.p. recombinant murine IL-33 on three consecutive days followed by ConA challenge. Liver damage was quantified by measurement of serum activities of alanine transaminase (ALT) and immune cell phenotype was determined by flow cytometry.

Results In ConA-induced immune-mediated hepatitis, severe liver damage was associated with elevated *Il33* and *Areg* tissue mRNA, as well as AREG serum protein levels. The release of IL-33 resulted in an increased frequency of hepatic ILC2 expressing the activation marker KLRG1, the effector cytokines IL-5 and IL-13 as well as AREG. In contrast, the expression of the co-inhibitory receptor PD-1 was downregulated. Moreover, the frequency of hepatic AREG+ Tregs was elevated. IL-33 treatment prior ConA application protected WT mice from immune-mediated hepatitis, enhanced ILC2 frequency and activation as well as expansion and AREG expression of ST2+ Tregs compared to ConA-treated mice. Furthermore, we induced immune-mediated hepatitis in *Areg*-/- mice to investigate its immuno-modulatory function in acute liver injury. Interestingly, these mice developed more severe hepatitis than WT mice. Hepatic ILC2 cells were more activated while the frequency of ST2+ Tregs was significantly reduced.

Conclusion The alarmin IL-33 plays a critical role in the alleviation of immune-mediated hepatitis. This immunoregulatory function might be driven by expansion and activation of ST2+ ILC2 and Tregs as well as induction of AREG expression in these cell populations.

5.6 HLA-B*27-restricted CD8+ T cell response against hepatitis B virus: viral escape as central mechanism of T cell failure

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Question Approx. 250 million people worldwide are chronically infected with the hepatitis B virus (cHBV) and are thus at high risk of progressive liver disease. Virus-specific CD8+ T cells play a major role in the control and clearance of HBV infections; however, the mechanisms of CD8+ T-cell failure in cHBV infection have only been partially elucidated, especially the role of viral escape. Since viral escape has been demonstrated in HCV and HIV infection in the context of HLA-B*27, we speculated that HLA-B*27 may also have a dominant role in driving viral escape in HBV infection.

Methods Through footprint analysis of 17 HLA-B*27 positive and 97 HLA-B*27 negative patients with cHBV genotype D infection and *in silico* epitope prediction, HLA-B*27-restricted HBV-specific CD8+ T-cell epitopes were identified and confirmed by *in vitro* antigen-specific expansion. The presence of CD8 + T-cell responses targeting the new epitopes was also tested in acute-resolving patients. Epitopes found through footprint analysis were analyzed for viral escape in cHBV infected patients. Phenotypical analysis was performed by MHC-class I tetramer-based enrichment.

Results 12 (5 by *in silico* prediction; 7 by footprint analysis) HLA-B*27-restricted HBV-specific CD8+ T-cell epitopes were identified. Epitopes identified by *in silico* prediction were dominantly targeted in both acute-resolving and cHBV infection, whereas epitopes identified by footprint analysis were preferentially targeted in cHBV. The *ex vivo* frequencies of HLA-B*27-restricted HBV-specific CD8+ T cells targeting conserved epitopes and variant epitopes were similar. After enrichments, the non-naïve T-cell population showed a PD1+CD127+ memory-like phenotype. The transcription factors TOX, TCF1 and Tbet were similarly expressed in HLA-B*27-restricted HBV-specific CD8+ T cells targeting conserved epitopes and variant epitopes. Interestingly, the expression pattern of Eomes and the inhibitory receptor KLRG1 was significantly higher in HBV-specific CD8+ T cells targeting conserved epitopes compared to CD8+ T cells targeting variant epitopes, indicating a more exhausted phenotype of CD8+ T cells targeting wildtype epitopes.

Conclusions cHBV and acute-resolving HBV infected patients exhibited a different CD8+ T-cell repertoire, which may have further implication on HBV chronicity. Further, viral escape may play an important role in HBV infection and may specifically affect CD8+ T-cell epitopes that are targeted during chronic infection only.

5.7 Liver-resident bystander CD8$^+$ T cells contribute to liver disease pathogenesis in chronic hepatitis D virus infection

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Question The hepatitis D virus (HDV) causes the most severe form of chronic hepatitis, often progressing to cirrhosis within 5-10 years. There is no curative treatment and the mechanisms responsible for the accelerated liver disease progression are unknown.

Methods We studied innate and adaptive immune responses in blood and liver samples of 24 HDV-infected patients and 30 uninfected controls by multiparameter flow cytometry in correlation with disease severity and stage.

Results The two major innate immune cell populations in the liver, MAIT cells and NK cells, were similarly affected by HDV infection. Compared to uninfected controls, their intrahepatic frequency was decreased in HDV infection, with greater prevalence of activated and degranulating cells in the liver compared to the blood. Most CD8$^+$ T-cells in the liver were activated memory or terminal effector memory cells, irrespective of HDV-specificity and viral escape, and the majority of activated and degranulated (CD107a$^+$) HDV-specific and total CD8$^+$

T-cells were liver-resident ($CD69^+CXCR6^+$). High-dimensionality reduction and Phenograph clustering of flow cytometry data identified an activated, memory-like, tissue-resident HDV-specific $CD8^+$ T-cell cluster with expression of innate-like NKp30 and NKG2D receptors. The size of this population correlated with liver enzyme activity (r=1.0). NKp30 and NKG2D expression extended to the total intrahepatic $CD8^+$ T-cell population suggesting global bystander activation. This was supported by the correlation between $NKG2D^+$ total $CD8^+$ T-cells and histologic activity index score, and the correlation of degranulated ($CD107a^+$) total $CD8^+$ T-cells with liver enzyme activity and APRI score.

Conclusion Inflammation and disease stage in HDV infection are driven by antigen-nonspecific activation of liver-resident $CD8^+$ T-cells.

Viral Hepatitis, Immunology

5.8 Inflammatory type 2 conventional dendritic cells contribute to cholangitis in mice

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Background The pathogenesis of primary sclerosing cholangitis (PSC) is not well understood. It is likely that dendritic cells (DCs) contribute to PSC pathogenesis. Because they are sentinel cells that reside close to bile ducts within the portal fields, they can readily recruit other inflammatory cells upon activation, and they are potent antigen-presenting cells that can effectively activate the adaptive immune system. However, the actual role of DCs in PSC is not clear.

Methods To characterise the contribution of DCs to PSC pathogenesis, we used three different mouse models that represent various aspects of PSC. 1) Acute 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC)-induced cholangitis; 2) *Mdr2* knock-out mice that spontaneously develop a bile toxicity-driven cholangiopathy; 3) K14-OVAp mice expressing an ovalbumin peptide in cholangiocytes that developed T cell-driven cholangiopathy following adoptive transfer of cognate OT1 T cells recognizing the ovalbumin peptide. Liver dendritic cells were characterised by applying a flow cytometry gating strategy that was previously developed by unsupervised analysis of flow cytometry data. Moreover, in the DDC model, DCs isolated from livers were subjected to single-cell transcriptomics using Cellular Indexing of Transcriptomes and Epitopes by Sequencing (CITE-Seq).

Results Cholangitis in all three mouse models was associated with strongly decreased relative numbers of plasmacytoid dendritic cells (pDCs) and conventional type 1 DCs (cDC1). In contrast, all three cholangitis models were characterised by a significant expansion of hepatic CD11c+ MHC II+ IRF8- CD172+ conventional type 2 DCs (cDC2), which compensated the decrease of the other DC populations, even leading to an overall increase in liver DCs in the DDC model and *Mdr2* knock-out mice. Single-cell transcriptomic data confirmed these findings and revealed that the remaining pDCs and cDC1 cells were transcriptionally rather unresponsive to cholangitis induction. In contrast, the expanded cDC2 population was strongly responsive to cholangitis induction and seemed to acquire a mature state with inflammatory activity.

Conclusions Cholangitis in different mouse models was associated with expansion of conventional type 2 DCs showing inflammatory activity. Thus, cDC2 cells and their inflammatory mediators might be a target for treatment of PSC.

5.9 Characterization of murine monocyte and macrophage subsets in immune-mediated hepatitis

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Introduction Monocyte and macrophage populations have been classified into pro-inflammatory M1 and anti-inflammatory/restorative M2 subsets based on expression of $Ly6C^{hi}$/CCR2 and $Ly6C^{int/low}$/CX_3CR1, respectively. However, whether these so defined M1 and M2 subsets particularly express genes associated with pro- or anti-inflammatory/restorative function in liver disease is not clear. Therefore, we aimed at analysing the gene expression profile of hepatic monocyte and macrophage subsets in immune-mediated acute and chronic liver inflammation.

Methods To induce acute liver injury, C57BL/6 mice received ConA and were analysed 8 h later. *Mdr2*$^{-/-}$ mice were used as a model of chronic liver inflammation. The PrimeFlow RNA assay was used for simultaneous analysis of mRNA and protein expression by flow cytometry.

Results In acute liver inflammation, the frequencies of $Ly6C^{hi}$ monocytes and macrophages as well as $Ly6C^{int}$ macrophages were increased. We determined high expression of CX_3CR1 by $Ly6C^{hi}$ monocytes and macrophages. $Ly6C^{int}$ macrophages were also characterized by expression of CX_3CR1, however, the frequency of CX_3CR1^+ $Ly6C^{int}$ macrophages was decreased compared to the $Ly6C^{hi}$ counterparts. Interestingly, gene expression profiling revealed an enhanced expression of both, genes associated with pro-inflammatory (*Tnf, Nos2, Il6*) and anti-inflammatory/restorative function (*Il10, Arg1, Chil3, Tgfb1*) in $Ly6C^{hi}$ monocytes and macrophages while $Ly6C^{int}$ macrophages were predominantly characterized by expression of genes associated with anti-inflammatory/regenerative function (*Il10, Chil3, Areg, Mmp9*). In 12 weeks old *Mdr2*$^{-/-}$ mice, the frequencies of $Ly6C^{int}$ and $Ly6C^{low}$ macrophages were increased while the frequency of $Ly6C^{hi}$ monocytes was decreased. The frequencies of CX_3CR1^+ $Ly6C^{low}$ macrophages and $Ly6C^{hi}$ monocytes were elevated. $Ly6C^{int}$ and $Ly6C^{low}$ macrophages showed elevated expression of genes associated with pro-inflammatory *(Nos2, Il6*) and anti-inflammatory/restorative function (*Il10, Chil3, Areg*). Moreover, *Il12b* and *Il10* gene expression were upregulated in all monocyte subsets.

Conclusion Chemokine receptor and gene expression analysis revealed that particularly the classical M1 phenotype characterization based on expression of $Ly6C^{hi}$/CCR2 is not appropriate for hepatic monocyte and macrophage subsets, which are characterized by high expression of CX_3CR1 and genes associated with both, pro- and anti-inflammatory/restorative function in liver injury.

5.10 IL-1β and TNFα enhance the CXC chemokine expression of Hepatitis C Virus infected cells

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Background & aims The Hepatitis C Virus (HCV) has developed several strategies to persist in infected cells without being cleared by the immune system. HCV intervenes in various cellular processes, e.g. via cleavage of host proteins. Transgenic mice expressing the viral protease NS3/4A are protected against LPS challenge due to elevated TNFα levels. Both TNFα and IL-1β are known to be upregulated in sera of HCV-infected patients. Recently, we were able to show that HCV further upregulates EGF-induced CXC chemokine expression.

The present study aims to analyse the influence of the cytokines TNFα and IL-1β on the CXC chemokine expression pattern of HCV infected cells.

Methods Cells harbouring the subgenomic replicon of HCV or HCV infected cells were stimulated with either IL-1β or TNFα or left untreated. Specific siRNAs or inhibitors were used and mRNA of CXCR2 ligands determined via qPCR. Activation of p65 and MEK1 was determined by Immunoblot. Binding of the NFκB subunit p65 to the CXCL8 promoter region was determined by chromatin immunoprecipitation (ChIP).

Results TNFα and IL-1β-induced expression of the transcripts encoding CXCR2 ligands is in part substantially enhanced by HCV. Knock down of the p65 subunit of the NFkB complex results in a significant decrease of basal as well as inducible CXCL8 mRNA expression. Consistently HCV significantly enhances p65 DNA binding to the CXCL8 promoter in response to stimulation with IL-1β as well as the IL-1β-induced phosphorylation of p65 at the serine residue 536. Interestingly, inhibition of EGFR likewise resulted in a downregulation of in particular the enhancing effect of HCV on IL-1β-inducible CXCL8 mRNA expression.

Conclusion The study demonstrates that TNFα- and IL-1β-induced upregulation of CXCR2 ligand mRNA expression is further enhanced by HCV. Strikingly, the data suggest *trans*-activation of the EGFR by IL-1β in HCV infected cells. Furthermore, the NFκB subunit p65 seems to be crucial for HCV-dependent further upregulation of TNFα- and IL-1β-induced enhancement of CXCR2 ligand mRNA expression. HCV further upregulates the IL-1β-induced induction of NFκB p65 phosphorylation at Ser536, indicating enhanced transcriptional activity of p65 due to increased translocation of NFκB p65 into the nucleus. In summary, the present study demonstrates that HCV significantly enhances IL-1β-induced CXCL8 mRNA in its host cell involving enhancement of NFκB DNA binding and activation of EGFR.

5.11 Soluble CEACAM1 induces suppressive Tregs and binds to CD5 in a heterophilic manner

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Introduction CEACAM1 (carcinoembryonic antigen-related cell adhesion molecule 1) is an immune checkpoint regulator that controls immunity via self- and heteroligation. Soluble CEACAM1 (sCC1) was originally discovered as a serum marker in human patients with obstructive and autoimmune liver disease. In murine autoimmune hepatitis (Concanavalin A-induced hepatitis), CEACAM1 induces Tregs, and enhances Treg stability. *Ceacam1*-/- mice exhibit hyperinflammation and persistence of liver injury. IL-2-dependent Treg induction requires signaling via CEACAM1-S (CEACAM1 with a short cytoplasmic domain), on CD4+ T cells. Contrary, the long CEACAM1 isoform (CEACAM1-L) inhibits immune signaling via two ITIMs. It interacts with co-inhibitory immune receptors (e.g. TIM-3) and limits T cell activation. CD5 is a Treg marker that is implicated in Treg signaling and stability.

Objectives The role of CEACAM1-ligation and sCC1 in hepatic immune regulation is unknown. The role of CEACAM1/sCC1 in co-cultures of CD4+ T cells and antigen-presenting cells (dendritic cells, DCs) for T cell activation and Treg induction is investigated. Furthermore, heteroligands for CEACAM1, such as CD5, are validated regarding CEACAM1-dependent Treg induction.

Materials & methods In sera from human patients and mice, CEACAM1 was detected in Western Blots. Cocultures from bone-marrow derived, FACS-sorted DCs and MACS-sorted T cells from WT and *Ceacam1*-/- mice were analyzed with and without addition of sCC1 in FACS. Binding of sCC1 to CD5 was identified after affinity chromatography in mass spectrometry and transfection studies. Suppressive capacity of Tregs was analyzed in a suppression assay.

Results In sera of patients with advanced PSC and in sera of WT mice sCC1 was detectable. In cocultures of CD4+ T cells and dendritic cells, addition of sCC1 inhibits production of IL-2 by CD4+ T cells and IL-12 by DCs, regardless of their CEACAM1 expression status. sCC1 strongly binds to DC-activated CD4+CD25+ T cells which results in phosphorylation of STAT5 and upregulation of Foxp3. Preliminary data showed that sCC1-induced Foxp3 cells were capable of suppressing proliferation of effector T cells. Furthermore, sCC1-binding to CD5 on CD5-transfected HEK293 cells was confirmed.

Conclusion Addition of sCC1 to CD/T cell cocultures supports Treg induction. Currently, the relevance of CEACAM1-CD5 interaction in the context of Treg homeostasis is under validation.

5.12 Deletions in the HBV s gene are associated with altered HBsAg composition in patients with HBsAg loss during NA therapy

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Question The hepatitis B surface antigen (HBsAg) consists of the components small (SHBs), middle (MHBs) and large (LHBs) HBsAg. Recently, we could show that HBsAg loss during nucleos(t)ide analogue (NA) treatment are preceded by decreases of the LHBs and MHBs ratios. In this study, we investigated the association between HBsAg composition and changes in their protein amino acids sequences within the HBV s gene during the process of HBsAg loss during NA treatment.

Methods Patients achieving HBsAg loss (n=14) during NA treatment and a population of patients without serologic response during NA treatment (including HBeAg or HBsAg loss; w/o SR; n=23) matched by HBsAg levels at baseline and duration of observation were retrospectively analyzed. HBsAg components were quantified in sera stored at -20°C collected before and during treatment. The complete HBV s gene was sequenced in all available serum samples of patients with HBsAg loss and in minimum three samples of patients w/o SR.

Results HBsAg loss occurred after a mean duration of 17 (12-53) months of NA treatment. In patients with HBsAg loss, LHBs and MHBs became undetectable at a mean of 3.9 (0-12) and 11.4 (0-53) months before HBsAg loss. In contrast, in patients w/o SR no significant changes in HBsAg composition or loss of LHBs and MHBs were detected. Of the patients with HBsAg loss, 50 % (n=7) showed a wildtype HBV s gene before and during NA treatment. In one patient a deletion of amino acids (aa) 58 to 100 occurred, which is located in the preS1-domain. Interestingly, in 43 % (n=6) a double deletion of aa 1-6 and aa 88-129 occurred, which are located in the start ATG of the preS1- (LHBs) and the preS2- (MHBs) domain. In contrast, 74 % (n=17) of patients w/o SR showed a wildtype HBV s gene in all analyzed samples and no patient achieved a double deletion of aa 1-6 and aa 88-129, respectively. Six patients w/o SR showed deletions in the preS1/2-domain, from which two include the start ATG of the preS2-domain, but showed no influence on MHBs levels in serum.

Conclusions Changes in the proportions of LHBs and MHBs that precede HBsAg loss are associated with the occurrence of a double deletion in the start ATG of the preS1- and the preS2-domain in many cases. The impact of those deletions on protein structure and the viral fitness of HBV needs to be studied more in detail.

5.13 Immunomodulation of NK cells by Ribavirin is driven by pSTAT-4 activation with increased IFN-γ secretion in HEV

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Background Hepatitis E virus (HEV) is one of the main causes for acute hepatitis globally. In most cases the infection is asymptomatic and self-limiting. Nevertheless, in some patient groups, such as immunocompromised organ transplant recipients, it can lead to severe courses. Natural Killer (NK) cells are an important part of the innate immune response and represent a first line of defense against viral pathogens by producing the antiviral cytokine IFN-γ and by natural cytotoxicity. Here we asked which impact NK cells have on a HEV infection in the context of Ribavirin (RBV), the current treatment for a chronic HEV infection.

Methods The human hepatoma cell line HepaRG was inoculated with a full-length HEV (MOI 0.5). After 7 days of culture the cells were co-cultivated with PBMCs from healthy donors for 24h at an E:T ratio of 1:1 or with RBV at a concentration of 500µM. The viral replication was measured by qPCR and NK cells were analyzed by flow cytometry.

Results Both the co-culture of PBMCs and the treatment with sole RBV decreased viral loads, however the combination had a synergistic effect. NK cells stimulated with RBV showed an increased expression of the activation marker CD38 (3439/3837; p<0.0001) as well as the activatory receptor NKp46 (3309/3501; p=0.0139) and a decrease of the inhibitory receptor TIGIT (1414/743; p=0.0005). Looking at NK cell functions, we found a reduction in cytotoxicity as assessed by expression of TRAIL (472/415; p=0.0087) and CD107a degranulation (258/212; p=0.0002), while the production of IFN-γ was significantly increased (1020/1394; P<0.0001). To identify the underlying mechanism, we investigated different cytokine stimulations such as IL-12, IL-15 and IL-18. Compared to the unstimulated controls the combination of IL-12/IL-15 (change 415; p<0.0001) and sole IL-12 (change 46; P=0.0127) were affected by RBV treatment while stimulation with IL-15, IL-18 or combined IL-12/IL-18 were not. Indicating, that RBV is acting on a distinct signaling pathway. Since expression of the IL-12Rβ1- and -Rβ2-subunits were not upregulated, we analyzed downstream events by phosflow staining. We found an increase in pSTAT-4 expression (450/478; p=0.0004) while other transcription factors such as pSTAT-1 and pSTAT-3 were not affected.

Conclusion In the context of an in-vitro HEV infection, RBV has an immunomodulatory effect on NK cells by increasing pSTAT-4 expression and subsequently enhanced IFN-γ production.

5.14 MAPKAPK 2 impedes hepcidin mRNA expression and maintains iron availability in response to bacterial endotoxins

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Background & aims The liver contributes to innate immunity towards pathogens by the synthesis of acute phase proteins (APP). These proteins minimize tissue damage and promote repair processes. They isolate and neutralize invading pathogens and prevent further pathogen entry. Some of them including the antimicrobial peptide hepcidin also maintain iron homeostasis. The expression of APP by hepatocytes is regulated by inflammatory cytokines and chemokines, which are released by non-parenchymal cells like macrophages and tightly controlled by the intracellular MAPKAP kinase (MK)2. So far, it is unknown, if MK2 plays a role for the synthesis of APP in the liver. Aim of this study is to reveal MK2-dependent mechanisms involved in the regulation of APP and in particular hepcidin expression.

Methods $MK2^{-/-}$ mice and wildtype mice were treated with 1 µg LPS per g body weight. Animals were sacrificed after incubation periods up to 48 hours. Serum and liver were prepared and subjected to transcript or protein analyses. For *in vitro* experiments murine hepatocytes were isolated and incubated with cytokines or with the supernatants of LPS-treated bone marrow derived macrophages (BMDM) and analyzed for transcript or protein expression.

Results Following LPS injection MK2 deficiency leads to an abrogation of chemokine and cytokine synthesis including IL-6, whereas transcript expression of only one APP, namely alpha-2-macroglobulin is abolished. Interestingly, serum levels of IL-1β are not diminished and expression of hepcidin mRNA is even more enhanced upon deletion of MK2 when compared to wildtype. This enhancement is independent from transcriptional regulation via STAT3, which is an essential mediator of APP expression and unaffected by MK2 in this content. Moreover, we observed defects in iron availability in $MK2^{-/-}$ animals.

Conclusion Regarding the dramatic collapsing effect of ubiquitous MK2 deficiency on LPS-induced synthesis of cytokines and chemokines, it is an interesting observation that in the liver MK2 is almost neglectable for the expression of most of the APP. However, MK2 impedes expression of hepcidin mRNA via a post-transcriptional mechanism that is controlled by the cross-talk of IL-6 and IL-1β, but independent from STAT3. We propose that this is important for the regulation of iron homeostasis *in vivo*. This observation should be of particular interest in view of the increasing number of potential indications for MK2 blockade as a therapeutic approach.

5.15 MAPKAPK 2 and 3 promote viral replication and resolution of intrahepatic myeloid-cell aggregates upon CMV infection

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Background & aims Cytomegalovirus (CMV) circumvents sterile immunity and establishes a state of latency from which the virus reactivates under immunosuppressive conditions causing substantial morbidity and mortality. Multiple organs are targets of CMV infection, however the liver represents a prime site of CMV replication and latency. We could recently demonstrate that an acute infection with murine (M)CMV induces activation of the MAPKAP kinase (MK)2, which is critical for the generation of a cytokine response to MCMV and drives an IFNAR1-dependent circuit that controls IL-10 production and limits aggregation of $CD11b^{+}$ myeloid cells in the liver. These intrahepatic myeloid-cell aggregates recruit cytotoxic $CD8^{+}$ T cells and enable their local expansion without causing severe liver pathology. Aim of this study is to understand the MK2-controlled mechanisms that regulate formation of these myeloid-cell aggregates and influence viral replication. Moreover, the role of MK2's homologous kinase MK3, previously unexplored in this context, should be clarified.

Methods Mice deficient in MK2 and/or MK3 were infected with MCMV and sacrificed after incubation periods up to 2 weeks. Serum, organs and immune cells were isolated and transcripts, proteins and viral titers were analyzed.

Results Our data suggest that beside MK2 its homologous kinase MK3 is also involved in controlling cytokine and chemokine release upon MCMV infection. In the $MK2^{-/-}$ liver, but also in the $MK3^{-/-}$ or $MK2/3^{-/-}$ liver the resolution of myeloid-cell aggregates involving $CD11b^{+}$ as well as $CD68^{+}$ cells is delayed, $Ly6C^{+}$ monocytes dissolve with delay only in the $MK2/3^{-/-}$ liver. Furthermore, the accumulation of proliferating $CD8^{+}$ T cells is enhanced when MK2 and/or MK3 are deleted. Viral replication in the liver as well as in the lung of $MK2^{-/-}$, $MK3^{-/-}$ and $MK2/3^{-/-}$ animals is diminished, whereas it is unaffected in the spleen.

Conclusion The results indicate that upon CMV infection the interplay of MK2 and MK3 regulates a molecular and cellular environment within the liver that facilitates viral replication, but also controls resolution of myeloid-cell aggregates and recruitment of $CD8^{+}$ T cells. Therefore, it is conceivable that viruses such as CMV exploit MK2- and/or MK2/3-dependent mechanisms to avoid local formation of immune cell aggregates and that this is part of a program involved in immune escape and possibly also establishment of latency.

5.16 Interactions with phospholipids and membrane remodeling by nonstructural protein 5A of hepatitis C virus

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Question Hepatitis C virus (HCV) is a global health threat with approximately 71 million individuals chronically infected worldwide. The nonstructural protein 5A (NS5A) of HCV plays a key role in the virus life cycle by (i) inducing formation of an RNA replication compartment, known as membranous web, and by (ii) controlling the switch from genomic RNA replication to viral particle assembly. NS5A consists of an N-terminal amphipathic helix (AH) intercalating into the cytosolic leaflet of cellular membranes, a folded domain 1 and domains 2 and 3 that are intrinsically unfolded in solution. The aim of the work was to gain insight into the molecular mechanisms underlying membrane sensing and remodeling by NS5A.

Methods We (i) expressed the full length wt protein (fl-NS5A), comprising its membrane attachment region, truncated protein constructs and proteins containing amino acid substitutions; (ii) analyzed their interactions with lipids in a protein lipid overlay assay (PLOA) and (iii) reconstituted fl-NS5A into liposomes and examined the liposomes by negative staining and transmission electron microscopy.

Results We show that AH is not the only membrane interacting domain of NS5A. We characterize a pattern of specific interactions of NS5A with phospholipids and show amino acids determining specificity of the interaction with phospholipids to localize in domain 1 of NS5A. We find NS5A-lipid interactions enhanced by domain 2 and 3, whereas mutagenesis of positively charged amino acid residues (K20A and K26A) in AH or deletion of AH and/or deletion of domains 2 and 3 from NS5A do not affect the specific interaction pattern of NS5A with phospholipids in PLOA. By using an *in vitro* liposome remodeling assay, we demonstrate that fl-NS5A senses lipid membranes followed by membrane curvature induction when reconstituted into liposomes.

Conclusions The data obtained elucidate novel aspects of NS5A function and unravel a distinct role of the protein domain architecture in the induction of the membranous web.

5.17 A cell culture model of high HBV replication in primary human hepatocytes derived from HBV infected humanized mice

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Question Primary human hepatocytes (PHHs) freshly derived from donors show higher innate immune responses than undifferentiated hepatoma cell lines but their availability and in vitro infection efficacy are limited. The **aim** of this study was to establish a model of in vivo infected human hepatocytes that are transferred to cell culture to achieve high levels of HBV replication intermediates in vitro.

Methods uPA SCID beige (USG) mice were transplanted with PHHs and infected with genotype D hepatitis B virions. After at least 12 weeks of infection when nearly all PHHs were stably HBV infected, hepatocytes were isolated by collagenase perfusion followed by repeated low-speed centrifugations. 4x10^5 cells/well were seeded on collagen-coated 24-well plates and cultured for 21 days in Williams' medium E with DMSO. Interferon-alpha-2a (IFN)/lamivudine treatment or HDV superinfection were started 1d after seeding. RNA and DNA were isolated from the same wells and HBV DNA, HBV pgRNA and ISG mRNA expression/GAPDH+RPL30 in cell lysates as well as HBV DNA, HBeAg and HBsAg in supernatants were determined by RT-PCR and ELISA.

Results The amount of PHHs isolated from USG mice, HBV DNA/cell and relative pgRNA expression remained stable up to 21d after seeding. High amounts of secreted HBV DNA (mean 1.8x10^8 cop/ml), HBsAg (mean 2.2x10^2 IU/ml) and HBeAg (mean 2.5x10^2 S/CO) could be detected (d14). Compared to untreated cells, treatment with 1000 IU/ml IFN resulted in significant reductions of intracellular HBV DNA/cell (p=0.013, 3.5-fold), pgRNA expression (p=0.024, 28.4-fold), HBeAg (p=0.048, 5.9-fold) and HBsAg (p=0.044, 5.3-fold) on day 14. As expected, LAM treatment reduced intracellular HBV DNA levels (d14: p=0.005, 3.9-fold). Vigorous induction of interferon-stimulated genes OAS1 and MxA remained stable in interferon treated cells between d7 and d21 (OAS: p<0.0001, 15.8-fold; MxA: p<0.0001, 55.15-fold; mean mRNA expression on d14 vs baseline). Coinfection of PHHs with genotype 1 HDV resulted in highly detectable intracellular HDV RNA but significant decline of intracellular HBV DNA (p=0.003, 7.31-fold) and relative pgRNA expression (p=0.017, 7.05-fold) on day 14 compared to early infection (d3).

Conclusion PHHs exposed to long-term HBV spreading in chimeric mouse livers can be stably cultured in vitro. Such novel cell culture model allows comprehensive drug and innate immunity studies in highly replicative HBV infected cells.

5.18 Characterization of in-vitro responses to immune modulation of HBV $core_{18}$-specific vs HBV pol_{455}-specific $CD8^{+}$ T cells

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Introduction and aim Currently several new therapeutic strategies including direct antiviral treatments and immunomodulatory options are being

developed aiming at functional cure (HBsAg loss) of chronic HBV infection (CHB). One option to modulate immune responses to achieve higher rates of HBsAg loss is to restore the exhausted T cell response. Recently, it has been reported that T cells targeting different HBV epitopes can be phenotypically and functionally different. However, the restoration capacity of these T cells has not been investigated. Thus, we studied the phenotype, function and the restoration capacity of HBV $core_{18}$- and pol_{455}-specific $CD8^+$ T cells from patients with CHB.

Methods Peripheral blood mononuclear cells (PBMCs) from 27 HLA-A2 positive CHB patients were isolated. $Core_{18}$- and pol_{455}-specific T cells were phenotypically characterized in 24 patients using peptide-loaded MHC I dextramer based enrichment. Furthermore, the function and the restoration capacity of epitope-specific T cells from 12 patients were measured after *in vitro* culture of PBMCs with $core_{18}$ and pol_{455} peptides with or without α-PD-L1 antibody, IL-12 and mitochondria-targeted antioxidant Mito-Tempo. Intracellular cytokine assay was used as functional readout.

Results $Core_{18}$- and pol_{455}-specific $CD8^+$ T cells showed diverse phenotypes with higher functionality and higher rate of memory-like phenotype in $core_{18}$-specific $CD8^+$ T cells. The expression of IFNγ in $core_{18}$-specific $CD8^+$ T cells significantly increased after using αPD-L1 antibody (IFNg median = 0.49 vs 0.98; p = 0.021). However, the expression of IFNγ in pol455-specific $CD8^+$ T cells did not significantly changed after using αPD-L1 antibody. In contrast, IL-12 significantly enhanced the IFNγ expression of pol_{455}-specific $CD8^+$ T cells (pol_{455} vs pol_{455}+IL-12 median = 0.04 vs 0.91; p = 0.0015) and not of $core_{18}$-specific $CD8^+$ T cells. The ability of Mito-Tempo in enhancement of $core_{18}$- and pol_{455}-specific $CD8^+$ T cells function was only observed in few patients in our cohort and there were no differences between the epitopes.

Conclusion Our data confirmed the previous studies that $core_{18}$- and pol_{455}-specific $CD8^+$ T cells are phenotypically and functionally different. Furthermore, we showed that the response of HBV-specific T cells to different immune modulations varies depending on the targeted antigens. These results have potential implications for developing immunotherapeutic approaches to achieve functional HBV cure.

5.19 Impact of intercellular communication between macrophages and hepatocytes on their responses towards CMV or LPS

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Background & aims Macrophages are key components of the innate immune response. With high plasticity they adapt their phenotype to distinct challenges. As first responders towards pathogens they express inflammatory cytokines and chemokines that regulate responses of the cellular microenvironment within the liver. Hepatocytes represent a prime site of viral replication upon cytomegalovirus (CMV) infection. So far it is unknown, how macrophages impede viral replication in hepatocytes or if at all. Contrariwise, the impact of hepatocytes on the macrophages" phenotype in case of CMV infection remains to be elucidated. In addition, similarities or differences between co-cultivated macrophages and hepatocytes after treatment with CMV or lipopolysaccharide (LPS) are not identified, yet. First, this study aims to evaluate the pathogen-induced molecular pattern that directs the intercellular communication between macrophages and hepatocytes. Second, the impact of macrophages on viral replication within hepatocytes is analyzed.

Methods Bone marrow derived macrophages (BMDM) and hepatocytes from wildtype mice were prepared. To analyze cytokine expression BMDM and hepatocytes were subjected to an *in vitro* co-culture system for 2 days. Afterwards cells were treated with UV-inactivated murine (M)CMV or LPS. To evaluate viral replication hepatocytes were infected with live MCMV and then co-cultivated with BMDM.

Results Following co-cultivation with hepatocytes BMDM express significantly increased Ly6G(C) and CD11c membrane proteins. Furthermore, these hepatocyte-directed BMDM show enhanced expression of pro-viral IL-10, but diminished expression of anti-viral IFN-β, IL-6 (also known as IFN-β2) and TNF-α after treatment with UV-inactivated MCMV. In accordance with this hepatocyte-directed BMDM also show enhanced IL-10 and diminished TNF-α levels upon stimulation with LPS, but in contrast to MCMV enhanced IFN-β and IL-6 expressions. Moreover, the presence of BMDM inhibits viral replication in MCMV-infected hepatocytes.

Conclusion Macrophages exert anti-viral effector functions on hepatocytes causing a reduction of viral replication in a co-cultivated *in vitro* system. However, the intercellular communication between hepatocytes and macrophages leads to a macrophage phenotype that promotes pro-viral, but reduces anti-viral characteristics. This indicates that hepatocytes impede anti-viral effector functions of macrophages, probably as a part of immune escape.

5.20 TLR4 KO MSC optimized protection in liver IRI via CXCR2/CXCL2-mediated crosstalk with Kupffer cells

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Background Bone marrow-derived mesenchymal stem cells (MSC) ameliorate liver injury caused by ischemia-reperfusion (IR). However, MSC have a short life cycle in applications and difficult homing. Although Toll-like receptor 4 (TLR4) is functionally expressed in MSC, its role in MSC function during liver IR is not clearly defined. Previous studies have found that TLR4 knockout can improve stem cell proliferation, we hypothesize that TLR4 knockout can promote the protective effect of MSC on liver IR

Method MSC isolation from the bone marrow of the Wild-type (WT) mice and TLR4 knockout mice, MSCs were injected 0.5h before live ischemia. After 1h ischemia and 6hrs reperfusion, poor liver reperfusion area, ALT/AST level, cytokines expression and release, polymorphonuclear leukocytes (PMN) recruitment were tested. We also co-culture the MSCs and Liver Non-parenchymal cells (NPC), then give them LPS or H2O2 stimulate to mimic in vivo experiment. Kupffer cells were abolished using liposome to test the Kupffer cells function in this process.

Result We found that TLR4-knockout (KO) MSC infusion during liver IR elicited more protection against ischemia and exhibits less inflammatory cell infiltration than in wild-type (WT) MSC. Kupffer cells induced liver-specific expression of the chemokine CXCL2 during IR, which was markedly enhanced in mice treated with TLR4-KO MSC. Loss of functional TLR4 in MSC markedly enhanced CXCR2 expression. This loss also strengthened MSC crosstalk with Kupffer cells mediated by CXCL2/CXCR2 chemotaxis and recognition, followed by increased IL-10 production in Kupffer cells.

Conclusions TLR4 changes in MSC induce gene expression and functional changes, and these alterations may represent a novel therapeutic strategy to improve the protective capacity of MSC.

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5.21 Decline of liver stiffness in patients with HCV under DAA therapy is associated with decrease of inflammation markers

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Background In patients with chronic hepatitis C (HCV) infection a fast decline of liver stiffness in non-invasive fibrosis measurement was described, even within the treatment period of therapy with direct-acting antivirals (DAA). Aim of this study was to evaluate the influence of inflammation on this in HCV patients under DAA therapy.

Methods We prospectively investigated change of liver stiffness by sonographic acoustic radiation force impulse (ARFI) at treatment initiation (base line, BL) and 12 weeks after end of therapy (SVR12) in 217 patients with HCV, treated with DAA therapy at our centre. In addition, demographic data, laboratory and virological parameters, cytokines and chemokines were obtained at these two time points, as well as at week 4 and at end of therapy.

Results ARFI values decreased from 1,86 m/s to 1,68 m/s (p = 0,01) which was most distinct in patients who had F4 fibrosis at BL levels (3,27 m/s to 2,37 m/s; p < 0,001). Initial elevated values of GOT and GPT, APRI score, FIB4 score, ferritin and IgG declined significantly (p < 0,001) under therapy but did not show any significant positive correlation (r < 0,1) with ARFI values. Albumin (p < 0,001) and thrombocyte count (p = 0,007) showed an increase and INR a decrease (p = 0,003; not in cirrhotic or F4 patients) under therapy without correlation to ARFI values.

The cytokines IL1α (p = 0,026), INFy (only in F4 fibrosis p = 0,043) showed an increase; TNFα (p = 0,031), IFNα2 (p = 0,036), IL10 (p = 0,005) and IP10 (p < 0,001) decreased under DAA therapy. Changes of TNFα (r = 0,54; p = 0,037) and IL10 (F1-F3 fibrosis, r = 0,32; p = 0,03) showed correlation with changes of ARFI values.

Conclusion In conclusion, our data demonstrates that ARFI values, fibrosis scores, systemic inflammation parameters and parameters of the liver function significantly improved under DAA therapy. In addition, we showed that not the fibrosis scores or platelet count correlate most with fast decline of ARFI values, but inflammation markers like the cytokines TNFα and IL10. ARFI measurement, commonly taken as a predictor of liver fibrosis, therefore had more correlation with inflammation markers than it had with markers of fibrosis.

5.22 Discovery of conserved HDV-specific CD8+ T-cell epitopes in HBV/HDV infection

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Background and aims Hepatitis D virus (HDV) super-infection of hepatitis B virus (HBV)-infected patients is associated with rapid progression to liver cirrhosis and hepatocellular carcinoma. The virus-specific CD8+ T-cell response is thought to have a major impact on the outcome of HDV infection. However, the HDV-specific T-cell epitope repertoire is only poorly characterized and mechanisms contributing to T-cell failure during chronic infection are not yet fully understood. Previously, we identified HDV-specific CD8+ T-cell epitopes in regions of the large HD antigen (L-HDAg) in which the HDV tolerates mutations to escape immune recognition. In this study, we aimed to discover T-cell epitopes that are located in conserved regions, in order to characterize the influence of these T cells on infection outcome.

Methods PBMC from 19 chronic and 15 resolved HBV/HDV infected patients were stimulated with an overlapping peptide (OLP) pool covering the L-HDAg. After *in vitro* expansion virus-specific CD8+ T cells were detected by intracellular cytokine staining. For positive responses, HLA restriction and epitope fine-mapping were defined. Tetramers were generated for detection of HLA-B*08:01 restricted HDV-specific CD8+ T cells.

Result Interferon gamma (IFNγ) secretion of CD8+ T cells in response to peptide pool re-stimulation was detected in 52.6 % of HDV RNA+ patient, whereas 66.6 % of HDV RNA- patients responded to at least one peptide pool. OLP-specific CD8+ T cells predominantly co-secreted IFNγ and tumor necrosis factor (TNF). In agreement with our previous findings, novel epitopes were nearly exclusively restricted by rare HLA-B alleles. As an exception, the more common allele HLA-B*08:01 restricted an epitope highly conserved in genotype 1. Responses against this epitope were detectable by tetramer staining *ex vivo*.

Conclusion We were able to further expand the known HDV-specific-CD8+ T-cell epitope repertoire. Importantly, we also identified an epitope that is located in a highly conserved virus region and restricted by a relatively common HLA class I allele. This epitope may allow further insights into the fate and function of HDV-specific CD8+ T cells in the natural as well as treatment-associated course of HBV/HDV infection.

5.23 Clinical establishment of a Laboratory Developed quantitative HDV PCR assay on the cobas 6800 high-throughput system

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Question Hepatitis Delta Virus (HDV) is a negative strand circular RNA virus with strong self-base-pairing and high diversity between the 8 known genotypes (GT). Both of these features pose a considerable challenge for diagnostic workflows and many available PCR assays are characterized by considerable run to run and inter-laboratory variability. The aim of the study was to establish a quantitative real-time PCR assay on the open channel of a fully automated PCR platform (cobas6800, Roche) offering improved consistency and reliability.

Methods A primer/probe-set targeting a highly conserved region upstream of HDAg (Coller et al., 2018) was selected and adapted for use on the cobas6800. The lower limit of detection (LOD) was determined using a dilution panel of the HDV WHO standard (n = 21/dilution). Linearity and inclusivity were tested by preparing 10-fold dilution series (GT1-7 cell culture derived; GT8 patient derived; n = 5/dilution). Patient serum samples containing a variety of bloodborne viral pathogens were subjected to the assay to confirm exclusivity (n = 60).

Results LOD of the HDV Utility-Chanel assay (HDV_UTC) was determined as 3.86 IU/ml (95 %-CI: 2.95-5.05 IU/ml) with a linear range from 10-10^8 IU/ml (GT1). A linear relationship was observed for all 8 genotypes with slopes ranging from -3.422 to -3.783 cycles/log and an R2 range from 0.984-0.997 (for GT 5-7 results are pending). Inter-run and intra-run variability was 0.3 and 0.6 Ct (3xLOD), respectively. No false-positive results occurred. To evaluate clinical performance, serum samples of 110 anti-HDV-Ab positive patients were analyzed using the HDV_UTC and the CE-IVD RoboGene assay. 58/110 and 49/110 samples were concordant

positive or negative, respectively. 3 samples (all Ct > 35.6) were only positive on the HDV_UTC assay (overall agreement 97,3 %). Quantitative comparison demonstrated a strong correlation (r: 0.922; 95 %-CI: 0.869-0.954; p-value: < 0.0001).

Conclusion We established a new quantitative and sensitive HDV PCR on the cobas6800 system. The use of highly automated, sample-to-result solutions for molecular diagnostics holds many inherent benefits over manual workflows, including improved reliability and reproducibility, as well as dynamic scaling of testing capacity. The assay showed excellent analytical and clinical performance, with inclusivity for all eight genotypes and a limit of quantification of 10 IU/ml, making it a sensitive new tool for HDV screening and viral load monitoring.

Z Gastroenterol 2021; 59: e1–e58

Namenverzeichnis/Authors' Index

Z Gastroenterol 2021; 59: e1–e58 |

Z Gastroenterol 2021; 59: e1–e58